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RELAPSING FEBRILE NODULAR NONSUPPURATIVE PANNICULITIS

A Report of a Case with a Review of the Literature

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THIS IS a disease aptly described by its name. It is characterized by recurring bouts of fever associated with the appearance of varying-sized subcutaneous nodules that are usually erythematous and may be painless or slightly tender. The nodules develop most commonly on the thighs and the arms, often on the abdomen and the back and occasionally on the lower parts of the legs. Over a period of from weeks to months the lesions regress, leaving shallow or atrophic areas where fatty tissue of the panniculus adiposus has locally disappeared, with the skin becoming subsequently attached to deeper structures. Sometime during the course there are subjective symptoms of varying intensity, including malaise, fatigue, fever, chills, generalized pains, nausea and headaches.

Pfeifer [1] made the first report in 1892 and pointed out that similar changes could be produced in the fat tissue by artificial means. In 1916 Gilchrist and Ketron [2] reported the second case. Weber,[3] in 1925, and Christian,[4] in 1928, in reporting the third and fourth cases, respectively, added the descriptive words by which this disease entity is still known. There were several more or less typical cases reported during the next fifteen years, until 1943, when Miller and Kritzler [5] reviewed 26 cases in the literature and reported 1 case. The patient was a 34 year old Jewish woman who had an unusually severe illness and died with acute lesions of relapsing febrile nodular nonsuppurative panniculitis. The autopsy was noncontributory to our understanding of the disease.

1. Pfeifer, V.: Deutsches Arch. f. klin. Med. **50**:438, 1892.

2. Gilchrist, T. C., and Ketron, L. W.: Bull. Johns Hopkins Hosp. **27**:291, 1916.

3. Weber, F. P.: Brit. J. Dermat. **37**:301, 1925.

4. Christian, H. A.: Arch. Int. Med. **42**:338, 1928.

5. Miller, J. L., and Kritzler, R. A.: Arch. Dermat. & Syph. **47**:82, 1943.

Since then reports of 8 additional cases have appeared. Larkin, de Sanctis and Margulis [6] reviewed the subject and added a case. The patient was a 23 month old white boy with a history of nodules on his ankles, which regressed into atrophic areas while he was in the hospital. Biopsy of these areas gave results compatible with this disease.

Another case was reported by Spain and Foley.[7] The patient, a 51 year old Irish man, entered the hospital with uremia. On the second day, subcutaneous nodules developed. The patient died of uremia, and at autopsy these nodules revealed a characteristic appearance; moreover, nodules were found not only in the subcutaneous fat but in the mesenteric and omental and pretracheal fat.

Friedman [8] reported another case with autopsy. A 23 year old woman had recurrent crops of painful red spots and elevated nodules, some of which ulcerated. There were associated fever, generalized aching, cough, fatigue and splenomegaly with prominent leukopenia. A culture of Staphylococcus aureus was obtained from the heart blood at autopsy, and the patient was believed to have died of staphylococcic septicopyemia. No changes were found in the visceral adipose tissue.

Arnold [9] reported a case: A 27 year old Caucasian woman had moderately tender, slightly raised, deep-seated nodules on the anterior part of the left thigh. Sulfadiazine and sulfathiazole were given without benefit, but sulfapyridine seemed specific, five relapses occurring when administration of this drug was discontinued and five remissions on readministration. The histologic picture of the biopsy tissue was typical.

Ives [10] reported a case in which a 53 year old white man showed a disease which clinically was compatible with the diagnosis of Weber-Christian disease, but in which no biopsy was made to confirm the diagnosis.

The case of a 23 year old soldier who had warm, erythematous, tender nodules, fever and malaise was reported by Zee.[11] Biopsy of the lesions revealed a typical picture. Penicillin therapy was tried, and a clinical remission followed, but whether this was spontaneous or due to the effect of the therapy was left undetermined.

A report of a case with autopsy was made by Ungar [12]: A 37 year old woman died from suppurative peritonitis. Tissues were taken both before death and at the postmortem examination, so that various stages

6. Larkin, V. de P.; de Sanctis, A. G., and Margulis, A. E.: Am. J. Dis. Child. **67**:120, 1944.
7. Spain, D. M., and Foley, J. M.: Am. J. Path. **20**:783, 1944.
8. Friedman, N. B.: Arch. Path. **39**:42, 1945.
9. Arnold, J. L.: Arch. Dermat. & Syph. **51**:94, 1945.
10. Ives, G.: J. Missouri M. A. **42**:409, 1945.
11. Zee, M. L.: J. A. M. A. **130**:1219, 1946.
12. Ungar, H.: J. Path. & Bact. **58**:175, 1946.

in the development of the nodules were observed and the various microscopic changes discussed. Characteristic lesions were present throughout the adipose tissue.

Mostofi and Engleman [13] reported a case of a 39 year old Filipino soldier who had recurrent symptoms for seven months with recurring nodules that regressed and left no scars. The patient died, and autopsy revealed widespread involvement of the fat tissue. Typical lesions were found in the subcutaneous, epicardial, peripancreatic, periadrenal, perirenal and mesenteric fat.

An interesting report of a similar condition in rabbits was made by Duran-Reynals.[14] He discussed the analogies with the disease in man.

REPORT OF CASE

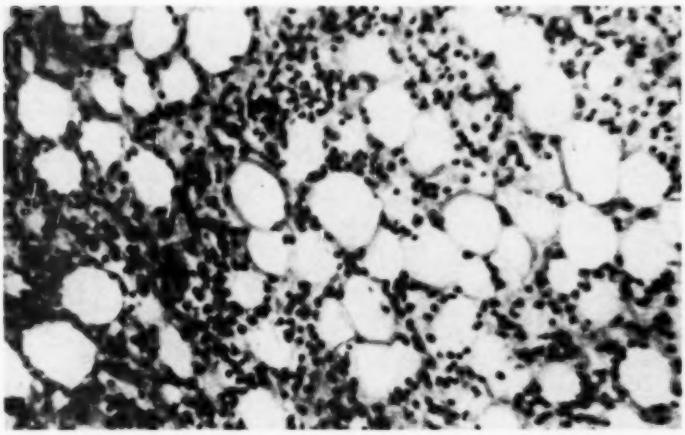
G. H., a 44 year old Negro man, was admitted to Cook County Hospital, Chicago, on July 21, 1947, complaining of small "bumps" which had been present beneath his skin for two months. In the two weeks preceding entry he had lost about 10 pounds (4.5 Kg.) and suffered from marked weakness and fatigability. In addition, he had felt feverish much of the time and occasionally had slight chills, followed by sweating. During the past few days he had noticed that the masses were receding in size. The history revealed that the patient had experienced three episodes of similar nodules and symptoms, the first eight years ago. Each episode had been ushered in with the appearance of nodules, which continued to increase in size for about two months and then to wane, taking approximately the same length of time to disappear. With the latter phase there had always been malaise, weakness, loss of weight and fever until the nodules disappeared. He had never received treatment. A history of syphilis and gonorrhea fifteen to twenty years before and also a history of malaria in 1934 while he was living along the Gulf of Mexico was obtained.

From the time of admission he had a continual low grade fever, the temperature reaching 101.4 F. Scattered over the arms, abdomen, chest, thighs and to a lesser degree the back were multiple nodules ranging from 1 to 3 cm. in size. These were firm in consistency and nontender to touch; a few were fixed to the skin, but most of them were movable. Lymph glands of the axillas and groins were enlarged. The liver was palpable 3 to 4 cm. subcostally; the spleen was not palpable. Physical examination gave otherwise normal results.

The initial white blood cell count was 1,800, and repeated counts never exceeded 3,950. Of these, 36 to 45 per cent were lymphocytes. The red blood cell count was 2,830,000 and 3,000,000 on two occasions, respectively. There was a moderate amount of anisocytosis, and occasional target cells and toxic lymphocytes were seen on a peripheral blood smear. The blood nonprotein nitrogen was 26 mg. per hundred cubic centimeters. The acid phosphatase was 0. Inorganic phosphorus was 5.1 mg. per hundred cubic centimeters. The total protein was 7.6 Gm. per hundred cubic centimeters, with a reversal of the albumin-globulin ratio (3.1:4.5). The Kahn test of the blood was negative. Urinalysis gave normal results repeatedly. Roentgen examination of the chest revealed no hilar or parenchymal abnormalities and a heart that was within normal limits. Roentgen examination showed the hands to be normal.

13. Mostofi, F. K., and Engleman, E.: Arch. Path. **43**:417, 1947.
14. Duran-Reynals, F.: Yale J. Biol & Med. **18**:583, 1946.

A biopsy of one of the nodes over the thigh revealed that the collagen connective tissue of the papillary and reticular layers of the dermis was dense, with slight edematous changes of the papillary layer. The blood vessels lying within that layer were surrounded by small numbers of lymphocytes. The loose, edematous connective tissue about some of the hair follicles and sweat glands was infiltrated by lymphocytes and occasional large mononuclear cells. Within the subcutaneous fat tissue there was a poorly circumscribed nodule which measured 10 by 4 by 3 mm. The nodule itself was composed of rather dense collagenous connective tissue, fat tissue and nests of simple sweat glands. The connective tissue about these sweat glands showed slightly bluish-staining cytoplasm suggestive of pseudomyxomatous degeneration of the connective tissue fibers, and was infiltrated by lymphocytes. The fatty tissue, on the other hand, showed an



Microscopic appearance of the lesion in the fat tissue of the skin.

extensive cellular infiltrate, including lymphocytes, plasma cells, large monocuclear cells and an occasional polymorphonuclear leukocyte, widely separating the individual fat cells and compressing some of them. In the fibrous connective tissue there were cellular infiltrates similar to that seen in the fat tissue, particularly about the blood vessels. The endothelium of these blood vessels was slightly swollen.

The patient remained in the hospital for eighteen days, during which time the nodules progressively decreased in size. During the first week of hospitalization the patient continued to suffer from weakness, fatigue, chilling and fever, but during the latter part of his stay these symptoms gradually diminished in intensity. He was released on August 7, and at that time he was feeling much improved and the nodules were barely palpable. The patient returned three weeks later to the outpatient clinic feeling entirely normal and without any evidence of the former nodules or of their previous locations.

COMMENT

To date 35 cases of relapsing febrile nodular nonsuppurative panniculitis have been reported. Of the patients, 25 were women and girls and 10 males. Of these patients, 6 have died from varied causes (only 1 definitely as a result of this disease), and lesions were examined at autopsy in 5 instances, thus adding considerably to knowledge of the disease. Beforehand it had been thought that the lesions were limited to the panniculus adiposus, but in 3 of the postmortem examinations [15] the inflammatory nodules were found not only in the subcutaneous fat but, in varying intensity, in the fat tissue of the abdomen and thorax.

Histologically, the fundamental feature is a cellular infiltration of the subcutaneous tissue, followed by edema and phagocytosis of the fat elements by macrophages, with a fibrous reaction occurring in later stages. A new concept of the histologic progression has been gained from 3 cases in which nodules in various stages of development or regression were obtained and examined. From these studies there have been described three main stages of the disease. First there is the early stage, in which the nodules have just appeared and are barely palpable. These reveal a moderate degree of infiltration, predominantly of leukocytes, between the fat cells. Then there is the large, well developed nodule, which probably represents the stage which has been most commonly described. In it the normal fat tissue lobule is replaced by many fat-laden macrophages plus lymphocytes, polymorphonuclear leukocytes and others. The third or late, atrophic stage is relatively acellular. Only a few lymphocytes and an occasional giant cell remain in the white fibrous tissue.

The cause of this disease is still obscure. Various chemical, thermal, infectious and mechanical injuries have been suggested but never proved to be causative. The use of iodides and bromides has preceded the onset of this disease in some instances and therefore has been regarded as a possible factor. Other factors, such as the injection of insulin or of hypertonic dextrose and sodium chloride solution, and avitaminosis, have also been mentioned. In the case reported here there was nothing in the past history which gave any clue to the cause.

Some believe this to be an infectious disease. In a fair number of the cases foci of infection have been found, most often in dental cavities and tonsils. The fact that nodules have been widely dispersed in fatty tissue might suggest a blood-borne agent. Repeated attempts to isolate bacteria from the lesions by smears and cultures, however, have been made without success. This made some consider the possibility of a virus infection. The character of the lesion, with destruction of fat and muscle, followed by infiltration and fibrosis, makes it similar to

15. Spain and Foley.[7] Ungar.[12] Mostofi and Engleman.[13]

many viral diseases. Others have entertained the possibility of its being an evidence of bacterial allergy. Arnold [9] made an interesting comparison with dermatitis herpetiformis, which also is a chronic relapsing disease, often aggravated by iodides and bromides, and which responds better to sulfapyridine than to other sulfonamides.

No treatment of this disease has been established as successful. In 1 case in which sulfadiazine and sulfathiazole had failed to produce any effect, the response to sulfapyridine made this drug seem specific, five relapses occurring on stopping the drug and five remissions on readministration. Penicillin was used in another instance,[11] but the part it played in the course of the disease is in question. In our case there were no acute symptoms and no treatment had been given, for the patient had improved by the time the diagnosis was certain.

SUMMARY

The case reported here, the thirty-sixth, agrees in the important clinical and histologic characteristics described in the literature for relapsing febrile nodular nonsuppurative panniculitis. The distribution of the nodules was typical, and the feature of recurrences was well demonstrated, the patient having entered the hospital in the fourth relapse. Low grade fever was present, but never the high, spiking temperature reported by some. Leukopenia was found in this patient, as has been noted in about one half of the cases. There were no pitted areas in the skin following healing, but this is understandable in a thin panniculus such as this patient had, in which the depression would be minimal and easily overlooked. By virtue of the repeated spontaneous remissions in this patient, real doubt was cast on the therapeutic value of some medications heretofore reported.

ONKOCYTIC ADENOMA OF THE SALIVARY GLANDS

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ONKOCYTES, first named by Hamperl,[1] are cells occurring in the ducts and acini of the salivary glands and in other locations. These cells had previously been studied by others. The word "onkocytoma" was introduced by Jaffé [2] for the tumor usually called "papillary cystadenoma lymphomatosum." Skorpil [3] recommended that this use of the term be dropped and that "onkocytoma" be used for the onkocytic tubular adenoma of the salivary glands. He further suggested "onkocytic adenoma" as a possible term for this type of tumor. Ackerman [4] used the term "onkocytoma" in the sense recommended by Skorpil and, like Skorpil, stated that it should be restricted to tumors located in the salivary glands.

Hamperl derived the name "onkocyte" from the Greek word ογκουσθαι, meaning "increase in bulk." It is to be noted that this Greek word is related to the Greek word ογκος, meaning "tumor" or "bulk," from which the English word "oncology" is derived. He found these cells in the ducts and acini of salivary glands and the serous, mixed and mucous glands of the tongue, the pharynx, the esophagus and the trachea. These cells are not found in these locations in persons under the age of 20 years. They become more prevalent in older age groups and can be found in practically every person over 60 years of age. Hamperl also felt that onkocytes should include similar cells appearing on rare occasions among the acinous cells and lining duct cells of the pancreas. He did not feel that their presence in the islands of Langerhans had been definitely established. He included the eosinophilic (Welsh) cells of the parathyroid glands, the Askanazy cells of the thyroid gland, the eosinophilic cells of Rathke's pouch, certain eosinophilic cells of the anterior lobe, the posterior lobe and the stalk of the pituitary gland and islands of characteristic cells of the lining

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1. Hamperl, H.: Ztschr. f. mikr.-anat. Forsch. **27**:1, 1931; Virchows Arch. f. path. Anat. **298**:327, 1936.

2. Jaffé, R. H.: Am. J. Cancer **16**:1415, 1932.

3. Skorpil, F.: Virchows Arch. f. path. Anat. **306**:714, 1940.

4. Ackerman, L. V.: Arch. Path. **36**:508, 1943.

epithelium of the uterine tube. Hamperl observed onkocyte-like cells in the lining epithelium of a few of the seminal tubules of an atrophic testis and in small nodules of parenchymal cells comprising portions of each of several hepatic cords of a cirrhotic liver. He did not feel that it had been established yet that onkocytes occur in the testis and the liver, because they had been observed in these two organs in only 1 case each. Hamperl [5] found cells somewhat resembling onkocytes among the mucous cells of the cardiac glands of the stomach in 2 cases. These cells differed from onkocytes in that the cytoplasm was more homogeneous and took a yellowish red rather than a red color with erythrosin-safranine.

Stout [6] noted onkocytes in the acini and ducts of the mucous glands of the bronchi. I have seen such cells in the ducts of the mucous glands in the vocal cords and the tonsillar capsule. Boeck and Schlagenhauff [7] found them in the lacrimal gland. Boeck [8] found them in a tumor of the lacrimal sac, and Radnot [9] has also seen them in the lacrimal sac. LaManna [10] described connective tissue cells with this appearance but probably was referring to macrophages with a granular eosinophilic cytoplasm. These occurred in the regions of breast where tissues had been removed for determination of carcinoma. Allegranza [11] expressed the belief that these cells eventually will be recognized in all tissues. It is to be noted that they have been observed only in adults or in pathologic tissues. The Welsh cells of the parathyroid glands are somewhat of an exception. They do not appear until late childhood but have been found as early as the seventh year. They probably have no secretory function. The onkocytic cells of the anterior lobe of the pituitary gland differ in appearance from the functional eosinophilic cells, being larger, darker and otherwise resembling onkocytes of other organs.

This heterogenous group of cells has been included under one name because of similarities in appearance. They resemble the cells of the tissue in which they are found but are larger and have a cytoplasm which is filled with fine, brightly eosinophilic granules or is itself brightly eosinophilic and finely honeycombed. Hamperl [1] expressed the belief that the honeycombing is a transitional stage in the development of the fully differentiated granular cytoplasm from the normal cell. The nucleus is often pushed toward the lumen or is in the center of the cell.

5. Hamperl, H.: Virchows Arch. f. path. Anat. **296**:82, 1936.
6. Stout, A. P.: Arch. Path. **35**:803, 1943.
7. Boeck, J., and Schlagenhauff, K.: Ztschr. f. Augenh. **94**:244, 1938.
8. Boeck, J.: Ber. u. d. deutsch. ophth. Gesellsch. **53**:299, 1940.
9. Radnot, M.: Ophthalmologica **101**:96, 1941.
10. LaManna, S.: Arch. per le sc. med. **66**:191, 1938.
11. Allegranza, A.: Ann. di biol. norm. e pat. **1**:242, 1946.

It resembles those of the surrounding cells or stains slightly darker and may appear smaller. Hamperl [1] expressed the belief that the apparent smaller size is due to a covering of the edges of the nucleus by the many fine cytoplasmic granules. Hamperl and others have stated that these cells divide by amitotic division. No normal mitoses have been seen except in the onkocytoma reported by Ackerman.[4] Hamperl expressed the opinion that these cells are not degenerative cells but represent a "further differentiation" or an "aging process." Allegranza [11] stated that the granules consist of proteins, sometimes linked with lipids. He expressed the belief that the honeycombed appearance of some of these cells is the result of the dissolving out of the fat in the preparation of paraffin sections. These granules sometimes take a faint fat stain. Skorpil [8] expressed the belief that in the case of onkocytoma which he reported this change was developing in the neoplastic cells, the onkocytic change occuring after the tumor's origin. Hamperl [1] also suggested the possibility of this type of change. The finding of smaller, denser-staining cells reported by several authors and the transition leading to disintegrating cells seen in my case suggest that this may be akin to a retrogressive change. It differs from the usual degenerations in that it is an apparently permanent, nonreversible process, is found to have been transmitted to daughter cells, following amitotic division, is usually connected with a loss of the ability to undergo normal mitosis and is related to the aging of the individual or to a pathologic process. Allegranza [11] has taken a somewhat similar view. None of the onkocytes are known to possess any function other than a mechanical one, such as lining ducts, although mucus was seen in some of the ducts of the tumor studied by me.

In view of the wide variation in types of tissue showing onkocytes, the retaining by onkocytes of some features of their original morphologic aspect and the observation presented in the foregoing paragraph as made by Skorpil, I feel that this phenomenon should be regarded as a change in the cells and not a change to a specific type of cell. Since use of the word "onkocyte" would imply that these are a specific type of cell, it would be preferable to speak of "onkocytic salivary duct cells" (for instance) rather than "onkocytes of the salivary ducts," and the term "onkocytic adenoma" should be used rather than "onkocytoma." The term "onkocytic adenoma" is also preferable because "onkocytoma" does not carry any implications as to the structure or the cancerous nature of the tumor. Although the use of "onkocytoma" has been opposed for papillary cystadenoma lymphomatosum,[12] that use is still widespread and causes confusion between these two tumor types. I feel that the term "onkocytic adenoma" should not be restricted to tumors of the salivary glands.

12. Hamperl.[1] Ackerman.[4]

Not only are onkocytes found in onkocytic adenoma but they are also the epithelial cell type in papillary cystadenoma lymphomatosum in many cases and in simple salivary gland cyst and mixed tumor of the salivary glands in some cases.[1] I have seen a small onkocytic cyst of the pharynx and have seen onkocytic areas in carcinoma of the parotid gland. In view of the occurrence of onkocytic cells and areas in other types of salivary tumors, a number of blocks should be studied in any case suspected of being onkocytic adenoma to exclude this possibility,

Cases of Onkocytic Adenoma

Author	Age, Yr.	Sex	Duration	Site	Shape and Size	Consistency	Color	Attachment to Skin	Follow-up
McFarland [13, 14]	71	M	3 yr.	Right parotid region	4.5 × 4 × 2 cm.	Soft solid	Dark brownish red	None	Recurrence 2 yr. later; died 6 mo. later of other causes
Blair and Olch [15]	..	..	.....	.....	.....	.....	.....	.....	
Huckel [17]	61	M	8 yr.	Parotid region	Plum size	Soft	Gray-red	.....	
Steinhardt [19]	67	M	.....	Right parotid region	.....	.....	.....	Well encapsulated	
Ahlbom [20]	59	M	4 mo.	Hard palate, fixed to bone	1.5 × 2 cm.	.....	.....	.....	No recurrence after 3 yr., 8 mo.
Gruenfeld and Jorstad [16]	66	F	3 yr.	Right parotid region	Globular; 3 × 4 cm.	Firm and elastic	Brownish red	None; encapsulated	
Skorpil [3]	83	F	.....	Parotid region	Size of child's fist	.....	Gray-brown	Well encapsulated	No recurrence after 2 yr.
McFarland [14]	74	F	Many years	Angle of left jaw	Pea size	.....	.....	.....	
Ackerman [4]	76	M	6 mo.	Left parotid region	2 cm.	Firm	Brownish red	None	No recurrence in 8 mo.
Lloyd [18]	59	F	.....	Preauricular region	3 cm.?	.....	.....	.....	
Author's case	53	M	18 mo.	Left parotid region	Round; 2.5 × 2.8 × 1.8 cm.	Moderately firm	Brownish gray	None	

although the diagnosis of "onkocytic adenoma" does not require that all cells be onkocytes. (See later comment on Skorpil's and Ahlbom's cases.)

Ten cases of onkocytic adenoma of the salivary glands have been described (table). Five additional cases of tumors have been considered by some authors as cases of onkocytic adenoma.

REPORT OF A CASE

A 53 year old white man seen at the New York Post-Graduate Medical School and Hospital had noticed a freely movable swelling of the left side of the face and angle of the jaw for one and one-half years but had had no distress, pain or tenderness. He was not sure whether there had been any recent growth.

The regional lymph nodes appeared normal. At operation the tumor was found embedded in the parotid gland. A branch of the facial nerve passed through it. The specimen was rounded and measured 2.5 by 2.2 by 1.8 cm. It was entirely encapsulated. The entire tumor was blocked for section.

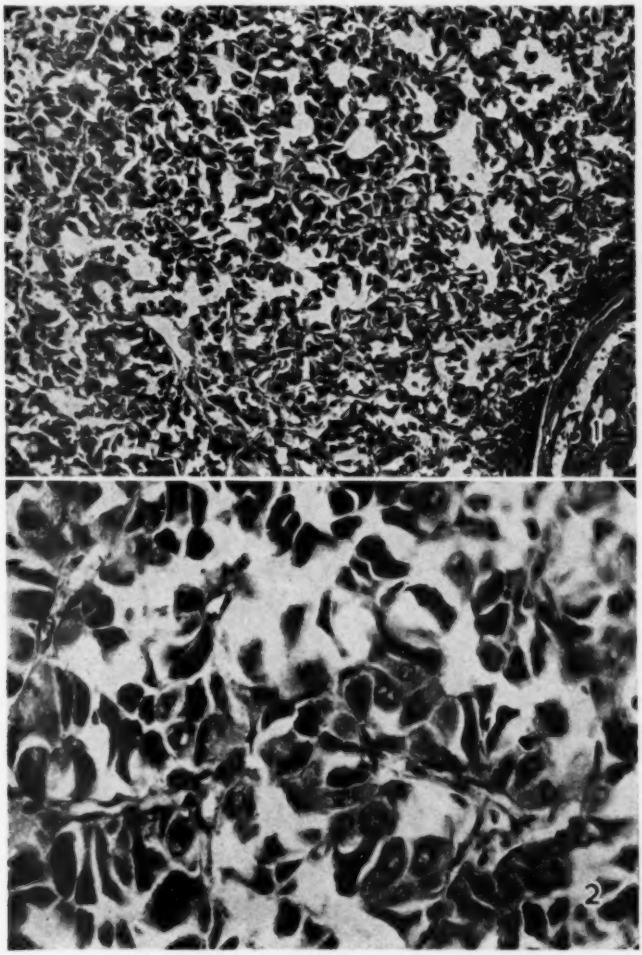

Fig. 1.—Onkocytic tumor. The general structure of the tumor is illustrated. Several ductlike structures are seen. × 100.

Fig. 2.—Thin fibrous septums separating masses of onkocytic cells and the darker, sclerotic cells, which are usually smaller. × 400.

Microscopically, the tumor was well encapsulated. Attached to the capsule was adipose and fibrous tissue containing in some areas groups of ducts suggesting atrophic remnants of the parotid gland. Many of these ducts were made up of

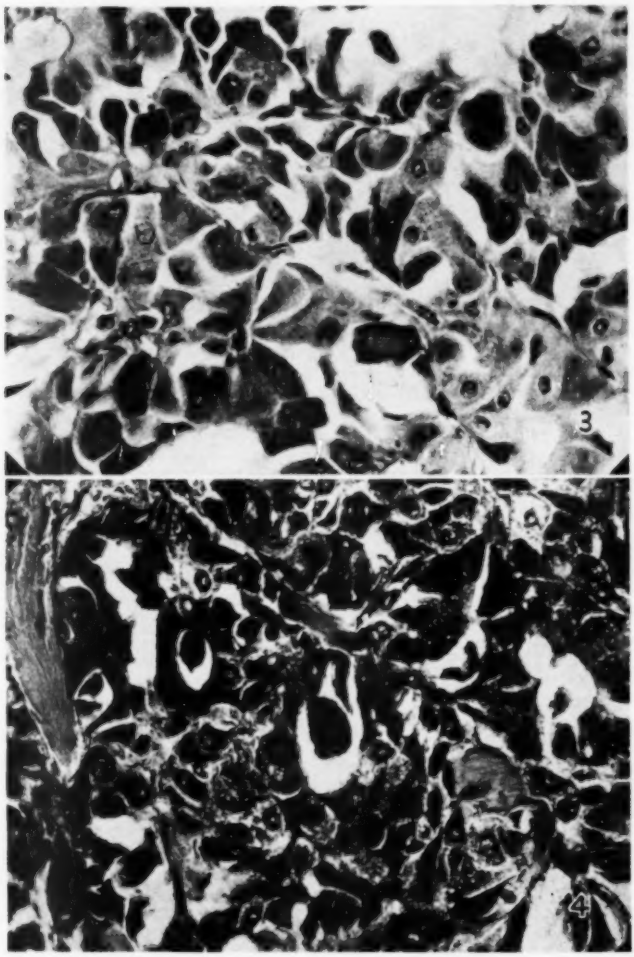

Fig. 3.—Onkocytic and sclerotic cells. One mass of secretion is seen. × 400.
Fig. 4.—Ductlike structures containing an opaque material, which stains with the mucicarmine stain. × 400.

onkocytes. There were areas of hemorrhage and of deposition of hemosiderin. One area showed compressed parotid tissue with degenerative changes in the acinous

cells. Small masses of tumor cells were seen in the capsule, but they appeared to be entrapped in it rather than invading it. One collection of approximately 18 cells was seen in a vessel. Their presence there was interpreted as an artefact. The tumor was divided into lobules of various sizes and shapes by hyalinized fibrous tissue septums of differing width. Narrower septums subdivided many of the lobules. The cells were from 7 to 22 microns in diameter. They were of irregular polygonal shape and formed fairly solid masses, which not infrequently contained groups of 8 to 17 high cuboidal or columnar cells forming ductlike structures. A few of these contained material which took a pale or moderate mucicarmine stain, but no mucus was seen intracellularly or outside these structures. A few of them were enlarged and showed flattened, compressed cells. The cytoplasm of the cells was made up entirely of fine granules which took a bright stain with eosin and an intense dark, opaque red with Masson's trichrome stain. The cells differed in the abundance of the granules. None of the cells had a foamy cytoplasm. Most of the nuclei were moderately vesicular and resembled those of the normal parotid gland. Many, however, were smaller and stained more heavily. These cells did not take the sudan IV stain. Scattered singly among the other cells and occurring in clusters were smaller ones in which the cytoplasm was darker and more homogeneous. The nuclei were also smaller and darker. That this was a degenerative process was suggested by the fact that the cells showed transitions in appearance leading to clusters of cells with disintegrating cytoplasm and pyknotic nuclei.

COMMENT

The first case of McFarland [13] was described in 1927 and referred to briefly in 1942.[14] McFarland's second case was not described anatomically, but in view of his wide experience, should be accepted. It is interesting, in view of Allegranza's [11] designation of Hürthle cell tumors as "onkocytic" tumors of the thyroid gland, that McFarland calls these tumors "Hürthle cell tumors of the parotid" and only as a subheading calls them "onchocytomas."

I have not been able to obtain the report of Blair and Olch,[15] but their case was accepted by Gruenfeld and Jorstad.[16] Ackerman [4] did not include it in his list.

The cases of Huckel [17] were not accepted by Gruenfeld and Jorstad and were not included in Ackerman's list. His first case only is accepted by Lloyd [18] and by Skorpil,[3] who studied the slides. In view of Skorpil's careful studies of this type of tumor, I feel that his judgment should be accepted. His paper shows a photomicrograph of Huckel's tumor that is typical in appearance. Hamperl accepted Huckel's 3 cases but with less careful study than Skorpil gave them.

13. McFarland, J.: Am. J. M. Sc. **174**:362, 1927.
14. McFarland, J.: Am. J. M. Sc. **203**:502, 1942.
15. Blair, V. R., and Olch, I. Y., cited by Gruenfeld and Jorstad.[16]
16. Gruenfeld, G. E., and Jorstad, L. H.: Am. J. Cancer **26**:571, 1936.
17. Huckel, R.: Ber. ü. d. deutsch. path. Gesellsch. **25**:342, 1930.
18. Lloyd, O. C.: J. Path. & Bact. **56**:699, 1946.

Steinhardt's [19] case was rejected by Skorpil but is accepted by Ackerman [4] and Hamperl.[1] His tumor had a characteristic gross appearance and microscopically was lobulated and had cells with a brightly eosinophilic cytoplasm. The centers of the lobules were made up of cells which were about the size of liver cells and had a granular cytoplasm. At the borders of the lobules the cells were larger and of loose structure with a netlike cytoplasm. The foregoing description is much like that of most of the other onkocytes reported. He also spoke of the cytoplasm as being "fibrillar," but when this is interpreted in the light of the rest of the description, it does not appear to indicate that the cells were strikingly different from other onkocytes. His description of the nuclei is fairly typical for onkocytes. The photomicrograph cannot be recognized as that of onkocytic adenoma, but the plate is dark. It shows cells in parallel rows. Steinhardt spoke of the cells of his tumor as being "transitional forms," such as Hemperl described in tracing the origin of the onkocytes from the cells of the salivary gland ducts, but his description seems typical enough to me to warrant inclusion of this tumor in the group designated as onkocytic adenoma.

Ahlbom's [20] description is too brief for evaluation, but the illustration is typical and the case is accepted by Ackerman [4] and by Skorpil.[8] Hamperl studied the slides and accepted this case, although he stated that some fields showed cells with honeycombed basophilic cytoplasm.

The remaining cases are typical (table, cases of Ackerman,[4] Gruenfeld and Jorstad [16] and Lloyd [18]). Skorpil's case is interesting in that it shows a poorly outlined area in which the cells are smaller and have a weakly basophilic cytoplasm. Typical onkocytes are scattered in this area, and the borders show a gradual transition of cell type to the onkocytes of surrounding areas. This structure led Skorpil to suggest that the cells changed to onkocytes after the formation of the adenoma.

CLINICAL FEATURES

The onkocytic adenoma has always behaved as a benign tumor, although in the first case of McFarland [14] it recurred after twelve years. In view of the characteristics of this type of tumor I feel that the second growth could have resulted from an additional focus of origin. Preoperatively the tumor is usually considered to be a mixed tumor.

CASES WHICH ARE NOT ACCEPTED AS INSTANCES OF ONKOCYTIC ADENOMA BY THE AUTHOR

Stohr and Risak [21] reported 2 cases which Ackerman [4] included in his list. Skorpil [8] did not make a definite statement but apparently did

19. Steinhardt, G.: Virchows Arch. f. path. Anat. **289**:624, 1933.

20. Ahlbom, H. E.: Acta radiol. (supp.) **23**:96, 1938.

21. Stohr, F., and Risak, E.: Arch. f. klin. Chir. **143**:609, 1926.

not consider them as cases of onkocytic adenoma. Hamperl did not mention these cases. The 2 cases are accepted by Gruenfeld and Jorstad.[16] The first one (Pr. Nn. 618/1911) is called a case of tubular adenoma, but no histologic description is given. The authors did not illustrate this case. The second case (Pr. Nn. 301/1925) was one of an encapsulated lobular tumor which microscopically had an acinous structure, with cells showing a marked similarity to the parenchymal cells of the normal serous gland. Some areas showed a granular cytoplasm. This tumor contained cavernous vascular structures. The photomicrograph is blurred. The cells in it look small and not too much like onkocytes. The authors did not mention the staining reaction of the cells. It is my feeling that no tumor should be classified as an onkocytoma unless its cells are known to take a bright eosinophilic stain, in addition to other characteristics; so I have not included these 2 cases.

Franssen's [22] case was accepted by Lloyd [18] but was not included in Ackerman's [4] list and Skorpil,[3] who had seen the slides, did not accept it, stating that its cells had a clear cytoplasm.

Lloyd stated that Leroux and Leroux-Roberts [23] reproduced a photomicrograph (their fig. 2) like Lloyd's onkocytic adenoma. It is true that Leroux and Leroux-Roberts showed several photomicrographs that suggest onkocytic adenoma, but their paper is a classification of the epithelial tissues of mixed tumors, and they did not give a complete description of their material, thus leaving the possibility open that all of these might be onkocytic areas of some other type of lesion.

ONKOCYTIC TUMORS OF OTHER ORGANS

Hamperl held that the eosinophilic tumors of the parathyroid glands, of which Castleman and Mallory [24] have reviewed 160 cases, is onkocytic adenoma. Hamperl and Allegranza held that the Askanazy tumor of the thyroid gland is onkocytic adenoma. Tumors of the latter name are called Hürthle cell tumors in this country. Most European authorities reject the name "Hürthle cell tumors" because they feel that the cells which Hürthle described are not the ones found in these tumors. The cells described by Hürthle were found in dogs in the interfollicle tissue of the thyroid gland. Similar interfollicle cells have not been found in man. The similar-appearing Askanazy cells do not occur in children but are found in adults and in pathologic conditions. They are found as epithelial cells lining follicles. Priesel [25] reported a tumor of the

22. Franssen, R.: Centralbl. f. allg. Path. u. path. Anat. **56**:113, 1932-1933.

23. Leroux and Leroux-Roberts: Bull. Assoc. franç l'étude cancer **23**:304, 1934.

24. Castleman, B., and Mallory, T. B.: Am. J. Path. **11**:1, 1935.

25. Priesel, R.: Virchows Arch. f. path. Anat. **267**:354, 1928.

pancreas which has been accepted as onkocytic adenoma by many authors, including Hamperl, who saw the slides. A small number of tumors diagnosed as onkocytic adenoma were accepted by Hamperl as having occurred in the posterior lobe, the anterior lobe and the pedicle, respectively, of the pituitary gland. In some instances adenoma of the bronchi was made of onkocytes.[6] Zippel [26] reported 1 case in which adenoma of the adrenal gland, and 1 case in which adenoma of the renal cortex, was made up of onkocytes. Boeck [8] reported a case of onkocytic adenoma of the wall of the lacrimal sac and Radnot [27] a case of onkocytic cyst of the same organ.

SUMMARY

Ten cases of onkocytic adenoma of the salivary glands and related glands of the hard palate have been reviewed and an additional one has been reported.

Certain tumors of the thyroid, parathyroid, pituitary and adrenal glands, the pancreas, the lacrimal sac and the kidneys have been reported to be onkocytic adenoma.

The possibility of the onkocytic change being a retrogressive one of unique type should not be discarded. The change should not be regarded as the development of a specific type of cell. The term "onkocytic adenoma" is preferable to "onkocytoma."

26. Zippel, cited by Allegranza.[11]

27. Radnot, M.: Ophthalmologica **113**:270, 1947.

EFFECT OF PROLONGED INTRAVENOUS ADMINISTRATION OF DEXTROSE ON BETA CELLS OF ISLETS OF LANGERHANS

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THIS is a preliminary report on studies being conducted to determine the function of the beta granules of the islets of Langerhans, with special emphasis on their role in diabetes mellitus. It is well established, on the basis of experimental data, that the beta cells are concerned in the elaboration of insulin. Furthermore, there is evidence that the beta granules are intimately related to insulin. In the work reported in this paper we endeavored to show the effects of prolonged intravenous administration of dextrose U. S. P. (d-glucose) on this specific granulation of the beta cells.

REVIEW OF LITERATURE

While the exact factors that stimulate the beta cells to elaborate insulin are not fully known, there is evidence that the level of the blood sugar is important. Anderson and Long [1] found that no secretion of insulin could be detected by assay of the fluid medium after an isolated pancreas had been perfused with blood low in glucose. When such a perfusion was made with blood containing large amounts of glucose, the perfusate indicated a great increase in secretion of insulin.

Houssay [2] observed that repeated or persistent hyperglycemia is an important factor in injuring the islets. He postulated that the hyperglycemia stimulates the beta cells excessively by inducing hyperfunction and hypersecretion; if long continued, this overstimulation leads to functional exhaustion and finally to injury and atrophy of the cells. Administration of sugar or of extracts of the anterior lobe of the pituitary gland or the thyroid gland causes elevation of the blood sugar.

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1. Anderson, E., and Long, J. A.: Endocrinology **40**:92, 1947.
2. Houssay, B. A.: Rev. med. panam. **1**:255, 1945.

Lukens, Dohan and Wolcott [3] expressed the belief that the hyperglycemia induced by anterior pituitary extract was the important factor in damaging the islet cells. They found that if the hyperglycemia caused by injections of anterior pituitary extract in cats was prevented by concomitant administration of phlorhizin or insulin, the diabetes mellitus and lesions of the islets usually resulting from the administration of the anterior pituitary extract did not develop.

In 1936 Jacobs and Colwell [4] maintained normal dogs on 50 per cent dextrose for as long as one hundred and sixty-eight hours, giving 0.7 to 4.5 Gm. of dextrose per kilogram of body weight intravenously per hour. Death ensued, and the chief abnormalities observed at autopsy were hemorrhages in the pancreas and the pituitary gland with some degenerative changes. However, they did not stain the islets with technics that would demonstrate the beta cell granules.

Woerner,[5] with continuous intravenous administration of dextrose, maintained hyperglycemia in guinea pigs and found mitotic activity, decreased granulation and hyperplasia of the beta cells. With higher levels of blood sugar, "exhaustion" of these cells was seen, manifested by degranulation or degeneration. He used the Lane-Bensley staining procedure, which we believe is less satisfactory than the Gomori [6] technic.

Gomori, Friedman and Caldwell [7] reported varying degrees of beta cell degranulation when transitory elevation of blood sugar was induced by a single intraperitoneal dose of dextrose; as normoglycemia returned, beta granulation was again observed. They offered the opinion that these histologic changes suggested functional activity of the beta cells, possibly related to the secretion of insulin as a response to the elevation of blood sugar.

Recently, Dohan and Lukens [8] reported that diabetes mellitus had apparently been induced in cats with repeated intraperitoneal injections of dextrose; the pancreases showed hydropic changes in the beta cells. However no mention of the beta granules was made.

METHODS

Normal dogs were used. After the animal had been anesthetized with pentobarbital sodium, a plastic (polyethylene) tube, 3 mm. in diameter, was inserted through the saphenous vein at the ankle and threaded up into the inferior vena cava.

3. Lukens, F. D. W.; Dohan, F. C., and Wolcott, M. W.: Endocrinology **32**:475, 1943.
4. Jacobs, H. R., and Colwell, A. R.: Am. J. Physiol. **116**:194, 1936.
5. Woerner, C. A.: Anat. Rec. **71**:33, 1938; **75**:91, 1939.
6. Gomori, G.: Am. J. Path. **17**:395, 1941.
7. Gomori, G.; Friedman, N. B., and Caldwell, D. W.: Proc. Soc. Exper. Biol. & Med. **41**:567, 1939.
8. Dohan, F. C., and Lukens, F. D. W.: Science **105**:183, 1947.

This plastic tube was connected to the rubber tube from the bottle of dextrose solution by means of a three way stopcock and a 15 gage needle. To immobilize this lower extremity, the sciatic and femoral nerves to this leg were sectioned, and the entire leg was splinted and wrapped securely with ace bandages and adhesive tape. This permitted the animal to stand and move about freely in the cage. To protect the rubber tube that led from the bottle of fluid to the dog's leg, this tube was passed through a sturdy, thick pressure hose that was securely incorporated in the leg wrappings at one end and fixed to the cage at the other end. Thus, kinks and strains on the more delicate tube that carried the fluid were obviated. The dextrose solutions were infused continuously, and the dogs were permitted to eat and drink ad libitum.

Vitamin B complex,[9] ascorbic acid (500 Gm.) and menadione sodium bisulfite U. S. P.[10] (4.8 mg.) were added daily to the intravenous fluids. Some 10 per cent dextrose in distilled water was given to the first dog, but because this concentration caused severe dehydration, the dogs that followed were given only 5 per cent dextrose in distilled water. Five per cent dextrose in sodium chloride solution was used occasionally in order to maintain fluid and electrolyte balance. Experiments were carried out successfully on 6 dogs; the seventh dog died twelve hours after the intravenous fluids were started and has not been considered in the data included in this paper. In each experiment, blood samples were drawn on a number of days for determination of sugar, carbon dioxide-combining power, fractional plasma proteins, urea nitrogen and chloride. The urine and the excreted sugar were not always measured because of technical difficulties. Each autopsy was made within one hour after death except as noted in the following pages. All tissues were fixed in Zenker's solution for hematoxylin and eosin stains. In addition, the pancreas was put in Bouin's fixative for Gomori[6] staining, and the liver was placed in absolute alcohol for Best's carmine stain for glycogen.

RESULTS

Duration of Experiments and Amounts of Dextrose Given.—It was possible, by the technic employed, to maintain continuous intravenous administration of dextrose for four days in 2 dogs (1 and 5), seven days in 1 dog (4), eight days in 1 dog (6) and nine days in 2 dogs (2 and 3). The amount of dextrose and the rate of injection for each dog are given in table 1. The amount of dextrose injected per day varied from a minimum of 50 Gm. (dog 5) to a maximum of 700 Gm. (dog 1). The total amount of dextrose given each dog varied from 425 Gm. (dog 5) to 2,250 Gm. (dog 3).

Blood Sugar Levels (table 2).—When the blood sugar was determined within an hour after starting injection of dextrose, the levels were high, viz., 607 mg. per hundred cubic centimeters in dog 1, 300 mg. in dog 3 and 306 mg. in dog 6. These initial high levels soon fell. This corresponds to the response usually obtained with the intravenous dextrose tolerance test, in which transitory initial rise is followed by normoglycemia in normal subjects. However, despite the fact that the animals were receiving large amounts of dextrose continuously, the value of the blood sugar remained within normal limits for three to seven days,

9. The preparation used was betasynplex (Winthrop) containing 20 mg. of thiamine, 10 mg. of riboflavin, 10 mg. of pyridoxine hydrochloride, 10 mg. of calcium pantothenate and 50 mg. of nicotinamide.

10. The preparation used was hykinone® (Abbott), which contains "not less than 49 per cent menadione ($C_{11}H_8O_2$)."

after which hyperglycemia developed again and persisted until the experiment terminated. During this maximum sustained hyperglycemia the value of blood sugar ranged from 154 to 1,000 mg. per hundred cubic centimeters.

TABLE 1.—*Amount and Rate of Administration of Dextrose*

Dog	Sex	Weight, Kg.	Day	Volume of Dextrose Solution per Day, Liters	Concentration of Dextrose Solution, per Cent	Gm. Dextrose per Day	Gm. Dextrose per Kg. per Day
1	F	17.7	1	5.0	5	250	14.1
			2	11.0	5	550	31.0
			3	6.0	5	300	39.5
				4.0	10	400	
			4	4.0	10	400	16.6
			Total	30.0	..	1,900	....
2	M	12.0	1	2.0	5	100	8.3
			2	2.0	5	100	8.3
			3	3.0	5	150	12.5
			4	5.0	5	250	20.8
			5	7.0	5	350	29.1
			6	4.5	5	225	18.7
			7	4.0	5	200	16.6
			8	4.0	5	200	16.6
			9	2.0	5	100	8.3
			Total	33.5	..	1,675	....
3	M	16.8	1	3.0	5	150	8.8
			2	3.0	5	150	8.8
			3	4.0	5	200	11.9
			4	4.5	5	225	13.4
			5	6.0	5	300	17.8
			6	11.0	5	550	32.7
			7	6.5	5	325	19.3
			8	4.0	5	200	11.9
			9	3.0	5	150	8.8
			Total	45.0	..	2,250	....
4	F	13.6	1	2.0	5	100	7.35
			2	4.0	5	200	14.7
			3	4.0	5	200	14.7
			4	7.0	5	350	25.7
			5	5.7	5	285	20.9
			6	4.0	5	200	14.7
			7	5.0	5	250	18.3
			Total	31.7	..	1,585	....
5	F	11.1	1	1.5	5	75	6.75
			2	3.0	5	150	13.5
			3	2.0	5	100	9.0
			4	2.0	5	100	9.0
			Total	8.5	..	425	....
6	F	11.3	1	2.5	5	125	11.1
			2	4.0	5	200	17.7
			3	4.0	5	200	17.7
			4	4.0	5	200	17.7
			5	1.0	5	50	4.4
			6	5.0	5	250	22.1
			7	3.0	5	150	22.1
				40 cc.	50	20	250
				400 cc.	20	80	
			8	600 cc.	20	120	10.6
			Total	24.54	..	1,395	....

Blood Urea Nitrogen, Carbon Dioxide-Combining Power, Chlorides and Plasma Proteins.—Except for dog 1, in which there was a marked decrease in the carbon dioxide-combining power (28 volumes per cent on the day before death) and dog 6, in which the plasma chlorides were reduced to 422 mg. per hundred cubic centimeters on the last day of the experiment, there were no noteworthy alterations

in blood urea nitrogen, carbon dioxide-combining power, chlorides or plasma proteins. These are shown in table 2.

Cause of Death.—In each experiment, the dogs fared well for the first two days, but after this, increasing lassitude and anorexia appeared. The condition of each dog was then one of gradual decline, but the exact cause of death was not apparent in all cases. In dogs 2 and 4 there was marked pulmonary edema, indicating overhydration.

Autopsy Observations.—Macroscopic Changes (table 3): In each instance autopsy was performed as soon after death as was practical; in most cases it was performed within three to four hours. The macroscopic findings in each

TABLE 2.—*Results of Chemical Examination of Blood*

Dog	Day	Blood Sugar, Mg. per 100 Cc.	Blood Urea Nitrogen, Mg. per 100 Cc.	CO_2-Combining Power, Volume per Cent	Chlorides, Mg. per 100 Cc.	Proteins, Gm. per 100 Cc.		
						Albumin	Globulin	Total
1	1	607*	10	55	...	...	...	5.2
	2	85	2	..	653	...	...	...
	3	159	10	28	622	...	...	5.5
	4	1,000	..	..	...	...	...	...
		782	..	..	...	...	...	...
2	2	100	..	47	617	...	...	...
	4	114	3	44	673	3.1	2.1	5.2
	5	135	..	..	...	...	...	...
	7	224	7	40	600	2.7	1.5	4.2
	8	254	..	..	...	...	...	...
3	1	300*	10	44	620	...	...	6.4
	4	140	5	..	667	3.0	1.9	4.9
	6	236	6	49	494	2.9	2.0	4.9
	7	130†	..	..	...	...	...	...
	9	203	..	..	...	...	...	...
4	2	102	4	39	654	3.5	2.3	5.8
	4	67	13	55	550	...	...	5.6
	7	176	7	59	556	3.5	1.8	5.3
5	2	83	8	44	670	3.9	1.7	5.6
	3	154‡	..	..	...	...	...	...
6	1	300*	..	..	612	...	...	7.6
	2	93	..	..	...	...	...	...
	3	113	3	43	582	3.4	2.5	5.9
	4	125	..	..	...	...	...	...
	6	106	5	41	494	3.6	2.1	5.7
	7	73	5	49	502	3.9	2.6	6.5
	8	333	5	48	422	2.7	3.0	5.7

* Blood was taken within one hour after start of intravenous injection of dextrose.
† Blood was taken one hour after intravenous injection of dextrose had stopped.
‡ Blood was taken during a convulsion.

of the dogs were not marked or uniform. In dog 1 the pancreas was congested, the kidneys showed cloudy swelling, the lungs were slightly congested and there were small hemorrhages in the spleen. In dog 2 there were ascites, hydrothorax and a rather marked hemorrhagic edema of the lungs. The pancreas appeared normal. In dog 3 slight congestion of the pancreas and slight swelling of the kidneys were observed, with a few hemorrhages in the lungs and beneath the serosa of the intestines. In dog 4 there were marked edema and small hemorrhages of the lungs and multiple petechiae of the colon, but the pancreas appeared normal. In dog 5, apart from an abscess and cellulitis at the site of the section of the femoral nerve, the findings were not noteworthy. Dog 6 was examined about five hours after death, and the essential findings were dehydration of all tissues, maceration of the skin and muscles of the leg in which the intravenous tube had been introduced, and central congestion of the liver.

TABLE 3.—*Observations**

Dog	Macroscopic	Microscopic
1	Autopsy within one-half hour after death Pancreas—congested Liver—friable, pale tan color Kidneys—cloudy, pale Lungs—slight congestion Spleen—small hemorrhages Large intestine—pasty red contents	Pancreas—Hematoxylin and eosin stain: only occasional islets intact; most show hemorrhagic necrosis, congestion, edema, and hemorrhage in lobules and interstitial tissue Gomori stain: complete degranulation of beta cells; most islets necrotic Liver—Hematoxylin and eosin stain: all liver cells completely clear and hydropic; no visible necrosis Glycogen stain: large amounts of glycogen shown Lung—congestion of septums, slight edema in alveoli, occasional bronchi filled with polymorphonuclear leukocytes Spleen—congestion and small areas of hemorrhage Kidney—congestion; small petechiae
2	Autopsy at 3 to 4 hours after death Body swollen and crepitant Lungs—hemorrhagic edema Ascites and plural effusion Pancreas—no changes	Pancreas—Hematoxylin and eosin stain: a few petechiae seen in the islets and acinous tissue; early postmortem changes Gomori stain: complete degranulation of beta cells of islets Liver—Hematoxylin and eosin stain: no significant abnormality; early postmortem changes Glycogen stain: no glycogen seen Kidney—mild interstitial edema; mild postmortem changes
3	Autopsy within 1 hour after death Pancreas—slightly congested Lungs—few hemorrhages, no edema Kidneys—slightly swollen Scattered serosal petechiae	Pancreas—Hematoxylin and eosin stain: a few petechiae in islets and in acinous and interstitial tissue; congestion Gomori stain: complete degranulation of beta cells in islets Liver—Hematoxylin and eosin stain: most lobules contain largely hydropic cells; others are normal; slight central congestion in lobules Glycogen stain: large amounts of glycogen shown Kidney—slight interstitial edema; otherwise normal Lung—subpleural petechiae in alveoli; otherwise normal Heart—normal except for occasional petechiae
4	Autopsy within 1 or 2 hours after death Pancreas—negative Lungs—marked edema and small hemorrhages Colon—petechiae and bloody contents	Pancreas—Hematoxylin and eosin stain: no abnormality seen Gomori stain: complete degranulation of all beta cells of islets Liver—Hematoxylin and eosin stain: a few lobules with some hydropic liver cells; others are normal Glycogen stain: moderate amount of glycogen seen at periphery of lobules Kidney—slight congestion and a few petechiae Lung—congestion of septums and edema in alveoli; a few petechiae seen
5	Killed by blow to head and autopsy performed at once Abscess and cellulitis at site of femoral nerve section No other gross changes	Pancreas—Hematoxylin and eosin stain: no abnormality seen Gomori stain: complete degranulation of all beta cells of islets Liver—Hematoxylin and eosin stain: no abnormality seen Glycogen stain: moderate amount of glycogen seen Lung—congestion of septums; slight atelectasis in some areas
6	Autopsy at not over 5 hours after death All tissues appear dehydrated Liver—nutmeg appearance Maceration of leg with intravenous tubes inserted No other gross changes	Pancreas—Hematoxylin and eosin stain: slight congestion in the islets; otherwise no abnormalities Gomori stain: complete degranulation of all beta cells of islets Liver—Hematoxylin and eosin stain: mild central congestion of lobules Glycogen stain: moderate amounts of glycogen seen at periphery of lobules only Lung—congestion of septums and edema in alveoli; dense exudate of polymorphonuclear leukocytes in bronchi

* Only the significant observations are tabulated.

Microscopic Changes (table 3): The routine hematoxylin and eosin stains of the pancreas showed no significant abnormalities of the acinous or islet tissues except in dog 1. In this animal, there were numerous diffuse hemorrhages throughout the parenchyma, and nearly every islet showed hemorrhagic necrosis. The islet cells had disappeared, and the area was replaced by hemorrhage. Only an occasional normal islet was seen. In the other dogs, only minimal changes in the islets were seen in hematoxylin-eosin preparations. With the Gomori stain, however, a complete degranulation of all the beta cells was found in every islet of each animal.

The lungs showed only edema and congestion. Occasionally there were erythrocytes in the alveoli, and a few bronchi showed accumulations of polymorphonuclear leukocytes.

The most marked changes found in the liver were those in dog 1, in which all the parenchymal cells were completely clear and hydropic, but there was no visible necrosis. Many of the cells appeared swollen, and except for the cell outline, no cell substance could be seen. These changes were found to a lesser extent in dog 3, in which a majority of the lobules showed largely hydropic changes. The livers of the other dogs were essentially normal except for varying amounts of minimal postmortem change. As determined by Best's carmine stain, glycogen was found in large amounts in the liver of dog 1 and in lesser amounts in the livers of the other animals.

No significant alterations were observed in the kidneys, the spleen or other organs.

COMMENT

It appears that prolonged hyperglycemia stimulates the beta cells excessively and that the overstrain exhausts the supply of insulin and causes degranulation. The only example of necrosis of the islet cells was seen in dog 1. The necrosis might have been due to overstimulation, but other explanations are possible. It is interesting that except for the initial transitory hyperglycemia within a few hours after the infusion of dextrose was begun, normoglycemia was maintained for three to seven days before persistent hyperglycemia developed. This might be regarded as a period in which the pancreas and other organs involved in carbohydrate metabolism could still meet the great demands placed on them; however, when this "overwork" was continued, islet "exhaustion" ultimately developed, and the blood sugar again became elevated. This might be regarded as an eventual decompensation of the functional ability of the islets to produce sufficient insulin, following the latent period in which the pancreas could compensate for the massive doses of dextrose and still maintain the normoglycemic state. Before such decompensation is manifested by hyperglycemia, degranulation occurs—apparently as an indication of pancreatic hyperfunction.

The cause of the hyperglycemia of diabetes mellitus is unknown, but the results of these experiments indicate that persistent hyperglycemia may in turn cause definite damage to the beta cells of the islets of Langerhans. This may be of significance in the management of patients with diabetes mellitus, in whom a prolonged state of

relatively poor control of the blood sugar may add to the damage of the beta cells which was present when the diabetes first became manifest.

SUMMARY

Dogs were given large amounts of dextrose, varying from 425 to 2,250 Gm., continuously by intravenous injection for as long as nine days.

After an initial transitory rise in blood sugar, there was a latent period of normoglycemia for three to seven days until sustained hyperglycemia developed.

In all instances the beta cells of the islets of Langerhans were degranulated. In dog 1, given massive amounts of dextrose to a total of 1,900 Gm. in four days, there was, in addition, hemorrhagic necrosis of the islets.

It is postulated that these changes are an indication of functional overstrain or "exhaustion" of the beta cells. This leads to the premise that the beta granules are related to the production of insulin.

The results of these experiments indicate that in clinical diabetes mellitus poor control of the blood sugar with resultant hyperglycemia may result in further damage to the beta cells and consequent increased severity of the diabetic state.

HISTOCHEMICAL DEMONSTRATION OF A LIPASE IN CARCINOMA OF THE LUNG

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OF A LARGE series of cancers, including both carcinoma and sarcoma, studied for lipase activity by Gomori, only 2, which were diagnosed as carcinoma of the esophagus and hepatoma, respectively, had this enzyme in their cells. A few unidentified stroma cells of an atypical seminoma were also shown to contain lipase.[1] No statement was made by Gomori as to the origin of the tumors which showed no lipase within their cells.

We have recently studied 6 cases of bronchogenic carcinoma in which wide variation of the lipase content of the tumors was found, which we report in the hope that our findings in this relatively unexplored field will be of use to students of this subject.

MATERIAL AND TECHNIC

With the exception of 1 case in which tissue was removed two hours after death at autopsy, the lungs which we studied were dissected within ten minutes after they were removed from the patient. Lipase was demonstrated in paraffin sections of acetone-fixed tissues by the Gomori technic.[1] The water-soluble ester of palmitic acid [2] was used, and the p_H of the substrate was adjusted to 7.3 to 7.4 with 4 per cent sodium hydroxide. The sites of lipase activity were stained by transforming the calcium palmitate formed into the corresponding lead salt and blackening with hydrogen sulfide. Control sections that received identical treatment except that they were not incubated in the substrate were routinely made. This was done in order to rule out calcium salts, melanin, lipochrome, hemosiderin and other pigments which could be confused with the lipase.

The remaining lung and tumor tissue were fixed in 4 per cent formaldehyde solution and stained with hematoxylin and eosin.

OBSERVATIONS

In areas of the lungs relatively uninvolved by disease the sites of lipase activity were similar to those found by Gomori.[1] The bronchial epithelium showed lipase activity in a patchy fashion. The epithelium of the ducts to the bronchial glands

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1. Gomori, G.: Arch. Path. **41**:121, 1946.

2. Tween 40 was used, obtained from the Atlas Powder Company, Wilmington, Del.

was usually strongly positive for lipase, and the cells of the bronchial glands showed both positive and negative staining cells. The attached septal cells of the alveolar walls were negative for lipase; desquamated epithelial cells were usually, but not always, positive. Muscle, cartilage and vascular and lymphatic tissue were negative. Where areas of fibroblastic connective tissue were seen, in several lungs faint diffuse staining was present.

The carcinomas observed were all of the epidermoid type and varied morphologically in minor degrees among themselves and in different areas of the same tumors. The following descriptions give the pertinent observations for each case:

Case 1.—J. L. B., aged 41, a white truck driver, had respiratory symptoms for six months. He died from a primary tumor which arose from the bronchus of the upper lobe of the right lung and from widespread metastasis to the brain, the liver, the adrenal glands, the kidneys, bones, the thyroid gland and lymph nodes. The autopsy was performed two hours after death. Sections showed the carcinoma in the lung and the liver to be composed of masses of undifferentiated moderate-sized cells with rather regular nuclei. The nucleoli were not prominent. Mitotic figures were infrequent.

No lipase activity was demonstrated in the carcinoma of the lung and the liver. Lipase activity was present in the macrophages of the lung adjacent to the carcinoma and in the hepatic cells of the liver adjacent to the carcinoma (fig. 1). The slides had been incubated in the substrate for eighteen hours.

Case 2.—J. G. G., aged 60, a white coal miner, had respiratory symptoms for two months. A sessile, fungating epidermoid carcinoma, grade 2, was found arising from a bronchus leading to the mesial inferior aspect of the lower lobe of the left lung. The tumor occluded the bronchus at one point. Sections showed the transition from normal bronchial epithelium to well differentiated cancer cells with good polarity. The outer cells of the invading tumor were columnar, while the cells in the interior of the tumor had squamous characteristics. In some areas the interior of the invading masses of tumor formed cystic areas filled with cellular debris. In other areas of the tumor jumbled masses of smaller cells with less cytoplasm invaded the bronchial wall. Mitotic figures were frequent in both varieties of cells. No metastases were found in the hilar lymph nodes.

The smaller cells of the tumor, the bronchial epithelial cells and the ducts to the bronchial glands showed lipase activity. The more differentiated invading cells revealed no lipase activity (figs. 2 and 3). The slides had been incubated in the substrate for forty hours.

Case 3.—C. S., aged 29, a white housewife, had respiratory symptoms for seventeen months. A sessile epidermoid carcinoma, grade 2, measuring 0.75 cm., in its greatest dimensions, was found arising from the bronchus of the upper lobe of the right lung. Sections showed small cells with dark angular nuclei, arranged in groups and strands. Mitotic figures were infrequent. The tumor had invaded the muscle of the wall of the bronchus but had not invaded an adjacent lymph node. No metastases were noted in the hilar lymph nodes.

No lipase activity was demonstrated in the neoplastic tissue, although adjacent bronchial glands and bronchial epithelium showed sites of lipase activity. The sections had been incubated in the substrate for eighteen hours.

Case 4.—I. C. W., aged 58, a white locomotive fireman, had a history of asthma and asthmatic bronchitis for many years. Two years prior to his pneumonectomy, an unexplained shadow was found by roentgenogram in the apex of the lower lobe of the right lung. A friable polypoid epidermoid carcinoma, grade 3, arising at the origin of the bronchus to the posterior lateral portion of the middle lobe of

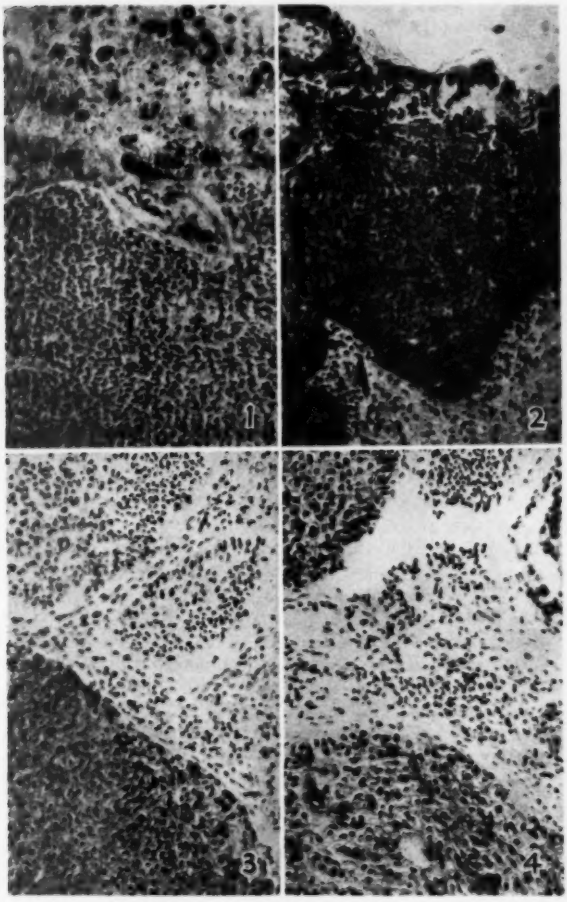

Fig. 1 (case 1).—A rapidly growing carcinoma in the lung that shows no lipase activity. The macrophages in the adjacent tissues show abundant lipase activity and appear black in the photograph. The nuclei of the cells are counterstained with hematoxylin.

Fig. 2 (case 2).—Both the carcinoma and the adjacent bronchial epithelium show lipase activity.

Fig. 3 (case 2).—This is the same carcinoma as the one seen in figure 2. The block of tissue was taken from the deeper portions of the tumor. Some portions of the carcinoma show sites of lipase activity while other areas of the carcinoma are without lipase activity.

Fig. 4 (case 4).—The carcinoma shows lipase activity. The lymphocytes appear dark in the photograph because they have been stained with hematoxylin.

the right lung was found. The polypoid growth obstructed the orifice of a small neighboring bronchus. The hilar lymph nodes showed extensive metastasis. Sections showed areas of spindle-shaped cells having prominent intercellular processes. Other areas showed cells of a large squamous variety with large, irregular nuclei.

In both varieties nucleoli were not prominent. Some of the invading masses of cells had a tendency toward a columnar cell arrangement in the outermost layer of cells, with inner cystlike areas filled with cellular debris. Other areas of the tumor showed cells with a complete loss of polarity. Mitotic figures were relatively infrequent. The metastases in the lymph nodes were composed of squamous epithelial cells.

There were sites of lipase activity in the neoplastic cells in all sections studied (fig. 4). The bronchial epithelium and the bronchial glands failed to show lipase activity. An occasional alveolar macrophage was positive. The sections had been incubated in the substrate for twenty-three hours.

CASE 5.—W. W. R., aged 68, a white farmer, had respiratory symptoms for six months. An epidermoid carcinoma, grade 2, arose from the wall of the bronchus to the lower lobe of the right lung, produced stenosis of a bronchus and was 10 cm. in diameter in its greatest dimensions. Microscopically, the tumor was characterized by well defined groups of squamous cells having moderate-sized and giant nuclei with multiple large nucleoli. Metastases were found in the hilar lymph nodes.

No lipase activity was demonstrated in the tumor cells, although the bronchial epithelium and the adjacent macrophages were strongly positive. The time of incubation of the sections was twenty-two hours.

CASE 6.—W. J., aged 55, a white coal miner, had respiratory symptoms for one year. A large, fungating, friable epidermoid carcinoma, grade 2, was found arising from the left bronchus. The tumor extended 6 cm. into the pulmonary tissue and obstructed several bronchi. No metastases were found in the hilar lymph nodes. Sections showed squamous epithelial cells with large prominent nucleoli invading the bronchial wall. A few areas were composed of smaller basophilic cells with much less cytoplasm. Mitotic figures were not frequent.

Lipase activity was demonstrated in the superficial squamous cells of the tumor. Other areas of squamous cells and the smaller basophilic cells of the tumor showed no lipase activity. Macrophages and the epithelium of the ducts of the bronchial glands showed lipase activity. The sections had been incubated in the substrate for twenty-two hours.

COMMENT

Gomori, in histochemical studies of lipase activity, demonstrated lipase within the cells of only 2 cancers of a large series. These 2 tumors arose from the mucosa of the esophagus and the liver cells, respectively. It is of interest that the normal cells of these tissues regularly contain this enzyme. Lipase activity has so far been demonstrated within tumor cells only in tumors arising from tissues which normally contain lipase.

SUMMARY

Using Gomori's technic, we studied 6 cases of bronchogenic carcinoma and found abundant evidence of lipase activity in 3 of them. No correlation of lipase activity and morphologic or biologic character of the tumor was possible.

HEALED DISSECTING ANEURYSM

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PHILADELPHIA

SINCE Shennan [1] reported on 300 cases of dissecting aneurysm of the aorta, the literature on this topic has become rather voluminous. A report of an isolated case, therefore, would not seem justified unless it exemplified some unusual feature such as that encountered in this case, which is reported because of the nature of the anomaly and the problems posed in its analysis.

J. E., a 58 year old Jamaican Negro, residing in Panama for thirty-six years, was brought to Gorgas Hospital on June 6, 1944, complaining of a sudden onset of epigastric pain and vomiting. He stated that he had previously been entirely well, never having undergone any illness, operation or injury. His parents and nine siblings were living and well.

On admission, the blood pressure was 140 systolic and 80 diastolic, with "normal" pulse and respiration rates and temperature. He did not appear in distress and was mentally clear. Heart, lungs and abdomen seemed normal. On admission laboratory examination revealed a hemoglobin content of 70 per cent, with 3,920,000 red cells. The white cell count was 13,900, 83 per cent of which were neutrophilic granulocytes. An electrocardiogram taken the following day revealed low T waves and slurred QRS complexes, but the tracing was not considered diagnostic. Roentgen examination of the chest and abdomen, intravenous urography and a barium sulfate enema disclosed no abnormality. Urinalysis revealed no abnormal findings.

For the first hospital week he complained of a steady periumbilical pain, which was occasionally relieved by antispasmodic drugs. A 2:1 heart block was noted, but otherwise subsequent electrocardiograms were unchanged. The blood pressure remained at 160 systolic and 80 diastolic. The impression at this time was "probably myocardial infarction." After two weeks he became symptom free and was discharged on July 31. At that time the studies were inconclusive, but discharge was effected because of available air transportation to the United States Army base where he was employed.

He was again admitted twenty months later, Feb. 21, 1946. At that time he complained that there had been mild nocturia for six months and severe headache for four days. The headache was followed by severe pain on the right side of the body, impaired vision and weakness, more noticeable in the left leg.

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1. Shennan, T.: Dissecting Aneurysms, Medical Research Council, Special Report Series, no. 193, London, His Majesty's Stationery Office, 1934.

At this time the blood pressure was 240 systolic and 150 diastolic, and the heart was enlarged to percussion. The rhythm was regular, and no murmurs were noted. The lung fields appeared normal, and the abdominal and neurologic examinations gave negative results.

The hemoglobin was 64 per cent and the red cell count was 3,740,000. The white cell count was 7,600, with 65 per cent neutrophils. The urine had a specific gravity of 1.016 and showed albumin (4 plus), with hyaline casts and red and white blood cells. The Wassermann test was negative. The blood nonprotein nitrogen was reported as 48.1 mg. and the creatinine 3.4 mg. per hundred cubic centimeters. Two days later the nonprotein nitrogen was 64.2 mg. A funduscopic examination was reported as showing a "grade III hypertensive retinopathy."

The blood pressure remained elevated, and the patient became progressively disoriented and comatose. March 1, 1946, the temperature rose to 106.2 F., and the blood pressure fell to 64 systolic and 50 diastolic. He died the same day. The clinical impression was "hypertensive cardiovascular disease with encephalopathy."

Autopsy (twelve hours after death).—Additional laboratory work on postmortem material revealed: specific gravity of the urine 1.017, with albumin (2 plus) and a few red cells and granular casts; blood nonprotein nitrogen 154.8 mg., urea nitrogen 120.0 mg., creatinine 8.2 mg. and glucose 137.0 mg. per hundred cubic centimeters. Wassermann tests of blood and spinal fluid were negative.

The external and general internal examinations disclosed essentially noncontributory conditions. The heart weighed 430 Gm., with a left ventricular wall measuring 18.0 mm. in thickness. The coronary arteries showed moderate patchy atherosclerosis. The aorta, being the point of interest, will be described in detail later. The lungs showed moderate edema, congestion and patchy areas of bronchopneumonia. The liver was slightly enlarged, weighing 1,750 Gm., and was moderately congested. The gallbladder and the biliary tract were not remarkable. The spleen appeared slightly fibrotic. The kidneys were slightly small, each weighing 140 Gm. and showed evidence of arterial and arteriolar sclerosis. There was moderate polyposis of the sigmoid colon. The remainder of the gastrointestinal tract, the pancreas, the adrenal glands and the genital organs were not remarkable. The brain weighed 1,350 Gm. and showed slight edema plus two small areas of ischemic necrosis, one in each lenticular nucleus. There was moderate cerebral arteriosclerosis.

The aorta showed slight atherosclerosis of the ascending portion. In the descending portion, 2.0 cm. distal to the ostium of the left subclavian artery there was an anomalous orifice, measuring 3.0 by 1.4 cm., and located on the left posterolateral aspect. This orifice communicated with an anomalous channel which traversed the length of the aorta along its left posterolateral aspect. Immediately distal to its origin, the anomalous channel showed a saccular aneurysmal dilatation, measuring 5.0 by 4.5 cm. This was traversed by pearly white delicate cords. A number of similar cords were noted along the course of this channel, running transversely and located at the inferior angle formed by the septum between the two vessels.

Arising from this accessory channel, in its thoracic portion, were a number of intercostal arteries, each completely patent. Similarly the left renal artery was seen to have its origin from the false channel, being at the same level as the normally located right renal artery. The terminus of this accessory aortic channel was the left common iliac artery, the origin of which could be considered similar to the origin of the left renal artery. At the point corresponding to the bifurcation of the aorta there was a communication between the two aortic channels which was covered by a delicate valvelike membrane that extended down into the left

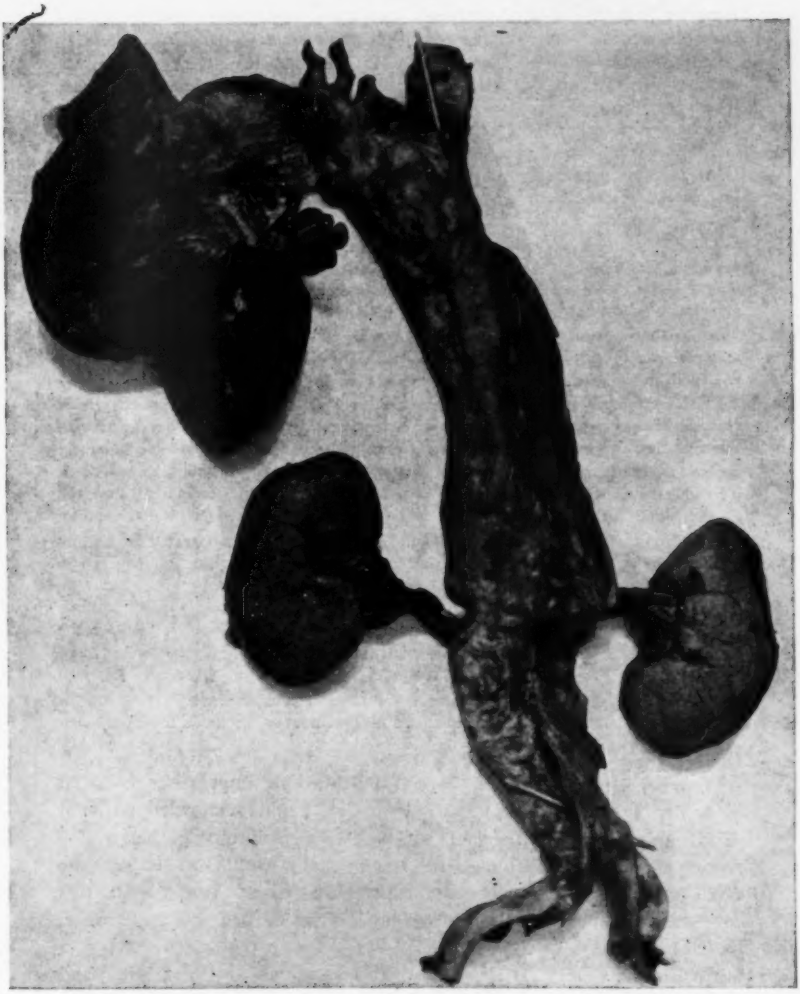

Fig. 1.—Anterior view: The normal aortic channel. Note the hypertrophy of the left ventricle and the similar, relatively normal appearance of the kidneys. Probes are inserted into the ostium of the anomalous channel and the communication leading to the left common iliac artery. (× 0.5).

common iliac artery for a distance of 1.2 cm. and attached on its medial wall. Thus, the circulation of the left common iliac artery was derived in the main from the anomalous channel, although some blood could come from the normal channel, since the valve was not completely obstructive.

Atheromatous plaques, some with superficial ulceration, were seen on the surfaces of both "aortas." The majority of these were in the anomalous channel, where they occurred in groups, particularly in the area near the renal artery. The two renal arteries, likewise the common iliac arteries, were of normal caliber, and each was similar in appearance to the other.

In general appearance the two aortic channels were of the nature of a double lumen tube, divided by a septum. Aside from the branches indicated, the major vessels arose in a normal fashion.

The kidneys were similar to each other in the changes and in the degree of change. The findings were those usually associated with hypertensive disease. There was moderate arteriosclerosis and a rather marked, though focal, hyaline arteriolosclerosis. Necrotizing arteriolar changes were not noted. In the areas of vascular involvement, the glomerular tufts showed changes varying from ischemia and collapse to fibrosis and hyalinization. Nearby, the convoluted tubules were enlarged and dilated. Foci of lymphocytes were present in the stroma.

The sections of the junction of the left renal artery and the anomalous aortic channel showed a direct continuity of all layers. There was moderate atherosclerotic deposit, with slight peripheral hemorrhage. The innermost medial fibers appeared slightly small and coursed irregularly, and there was moderate fibrosis. The internal elastic lamina was seen only in the renal artery portion. It appeared to end blindly at the ostium. There was lymphocytic streaking in the adventitia.

Histologic studies were made of the aorta, the anomalous channel and the septum between, using hematoxylin-eosin, Van Gieson and elastic tissue stains. These revealed similar components in each vessel, namely, intima, media and adventitia. The septum was composed of medial fibers, bordered on each side by intima. Atheromatous changes were prominent in both vessels. The atheromas of the anomalous channel were bordered by small hemorrhages. There was no evidence of cystic medial necrosis. The medial fibers forming the internal half of the wall and septum of the anomalous channel were small and irregularly formed. In both vessels there were linear collections of lymphocytes in the media and perivascular collections in the adventitia.

On completion of the autopsy, the immediate impression was that of healed dissecting aneurysm. However, rapid acknowledgment of disturbing features was made. It was thought probable that a dissecting aneurysm involving the renal artery would so impair the renal circulation that gross manifestations would result. In this case the two kidneys were remarkably similar in size and appearance, without evidence of infarctions. Thus, the situation of having a normal renal artery arising from an anomalous aortic channel and supplying an unremarkable kidney suggested the possibility that the original impression was incorrect. It seemed, at that time, improbable that an aortic dissection could occur across, rather than along, a vessel such as the renal, so as to reimplant this artery in the new channel without embarrassing the circulation.

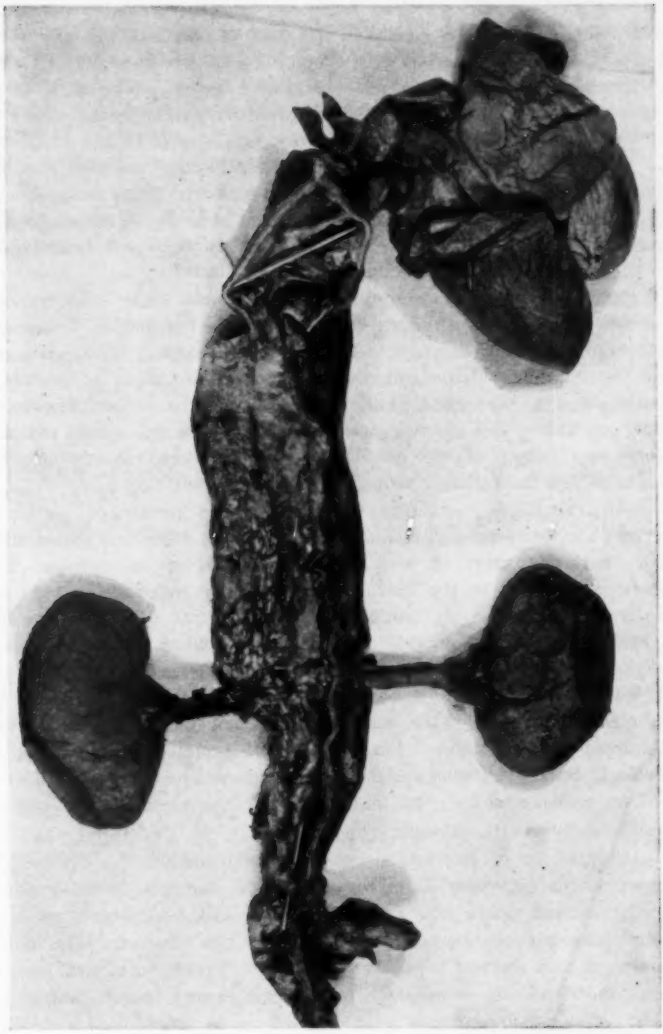

Fig. 2.—Posterior view: The anomalous aortic channel. Note (and compare with figure 1) the origin and the caliber of the left renal artery and the degree of atherosclerosis. Probes are inserted into the saccular dilatation and beneath the membrane covering the ostium of the left common iliac artery. (× 0.5).

A review of the standard textbooks and the available literature was noninformative. A consultation among members of the laboratory and hospital staffs resulted in the opinion that we were dealing with a congenital anomaly, a double aorta of a lower phylogenetic form resulting from failure of fusion of the primitive paired aortas. Several authorities, consulted by letter, concurred in this opinion.

With the apparent presence of an extremely rare anomaly, publication was considered at that time but was deferred because there still remained a certain element of doubt as to the tenability of the diagnosis, particularly since many early pathologists misinterpreted healed dissecting aneurysms as congenital lesions.

Almost at the same time as this case was under observation, Cassidy and Pinniger [2] were reporting a case of healed dissecting aneurysm in which the right renal and testicular arteries arose from the false channel. No mention was made of the caliber of the renal artery or of a comparison of the vessels on the two sides. However, the right kidney did show several gross infarcts, which would indicate some impairment of the circulation. This report recently became available and immediately reopened the discussion.

Once again the literature was combed for similar or pertinent reports and correspondence was undertaken with numerous authorities. The literature surveyed was, as before, noncontributory, and the opinions ventured by the consultants were of an indefinite and inconclusive nature. The predominant opinion was that this did represent a healed dissecting aneurysm, despite the anomalous features. However, a congenital lesion was not considered as being entirely excluded. It was decided, therefore, to consider this as an unusual type of dissecting aneurysm. The causation was not clear. Some of the histologic features suggested a diagnosis of syphilis, despite negative serologic tests. An arteriosclerotic factor must be considered. There was no evidence of the cystic medial necrosis most commonly reported in conjunction with dissecting aneurysms.

It might be of interest to consider and compare the degrees of atheromatous degeneration in the two aortic channels. That present in the normal aorta was moderate in amount. However, as was noted, the plaques present in the anomalous channel were more numerous and showed a peculiar grouping. From the clinical standpoint the dissecting process was of at least twenty months' duration. Thus, it would appear that a greater degree of atheromatous deposit can occur in a newly formed vessel in a relatively short time than had developed during the total life of the patient in a normal vessel of similar caliber, but bearing a greater burden.

2. Cassidy, M., and Pinniger, L. L.: Brit. Heart. J. **8**:130, 1946.

It was shown that the last twenty months of life were accompanied clinically by rapidly increasing hypertension. Some correlation between hypertension and atheroma is a well known concept (Boyd [3]). It is conceivable that a greater degree of atheroma could form in a vessel wall which was not previously normal, if one is to consider the possibility of a congenital lesion, or in the walls of a vessel produced as a result of some destructive process such as a dissecting aneurysm.

The mechanism by which the renal artery was reimplanted without embarrassing the circulation, leaving an essentially normal vessel, is not clear. It is of interest, however, to consider to what extent the healing process can occur in the dissecting aneurysm.

SUMMARY

An unusual case, most probably one of a healed dissecting aneurysm of the aorta, is reported.

An indication of the problems encountered in the analysis of obscure lesions has also been briefly presented.

3. Boyd, W.: A Textbook of Pathology, Philadelphia, Lea & Febiger, 1943, pp. 394 and 397.

ISOALLERGIC ENCEPHALOMYELITIS PRODUCED IN GUINEA PIGS

Via Intramuscular and Intraperitoneal Injection of Antigen

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THE PROBLEM of the genesis of postinfectious encephalitis, which first arose from clinical observation of encephalomyelitis following antirabies treatment and postvaccinal encephalitis, prompted some of the initial investigations on the relationship of allergy to the nervous system. From a clinical standpoint several authors had already advanced the theory of an allergic origin of certain neurologic inflammatory diseases. Using various types of brain suspension, Rivers, Sprunt and Berry,[1] Rivers and Schwentker [2] and Ferraro and Jervis [3] were able to produce in rabbits and monkeys an encephalomyelitis characterized by perivascular cellular infiltration and demyelination. Jervis, Ferraro and the Kopeloffs [4] succeeded, on the other hand, in determining in the brain of the monkey, as a result of repeated sensitizations with egg white, the Arthus phenomenon at the site of the intracerebral injection of the antigen, and at a distance, in the same brain, a form of encephalomyelitis closely resembling histologically the one obtained by Rivers.

One of us, Ferraro,[5] in 1944 described histologic changes occurring in the brain of a patient who had died of scarlet fever encephalitis and related these changes to an allergic brain reaction. In addition, on the basis of neuropathologic changes in human demyelinating diseases, which he compared with his experimental results, he emphasized the

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1. Rivers, T. M.; Sprunt, D. H., and Berry, G. P.: J. Exper. Med. **58**:39, 1933.

2. Rivers, T. M., and Schwentker, F. F.: J. Exper. Med. **61**:689, 1935.

3. Ferraro, A., and Jervis, G.: Arch. Neurol. & Psychiat. **43**:195, 1940.

4. Jervis, G. A.; Ferraro, A.; Kopeloff, L., and Kopeloff, N.: Arch. Neurol. & Psychiat. **45**:733, 1941.

5. Ferraro, A.: J. Neuropath. & Exper. Neurol. **3**:239, 1944.

relationship between demyelinating diseases and allergic reactions of the brain.[6]

With the Rivers brain suspension technic many months were required to determine experimental encephalomyelitis. Freund and McDermott [7] devised a technic in which adjuvants were added to the antigen, a modification which shortened the period of incubation for the production of the inflammatory reaction in the brain and spinal cord.

Freund's technic was first used in monkeys by Morgan [8] and, independently, by Kabat, Wolf and Bezer.[9] We [10] succeeded in reproducing in monkeys chronic aspects of the encephalomyelitis by using small doses of the antigen. Acute encephalomyelitis has been reproduced in rabbits by Morrison [11] and in guinea pigs by Freund, Stern and Pisani,[12] the Kopeloffs,[13] Alvort,[14] Jervis and Koprowsky [15] and ourselves.[16]

In 1946 the Kopeloffs [17] reported the presence of antibrain antibodies in the serums of monkeys treated with many injections of alcoholic extract of sheep brain incorporated in water-in-oil emulsion, following Freund's technic.

The original theory advocating specificity in the sense of organ selectivity rather than organ species preference in the production of the encephalomyelitis seemed to receive confirmation. In other words, an emulsion of either homologous or heterologous brain is able to produce an inflammatory reaction in the nervous system of the host. Antibrain antibodies resulting from the injection of the brain emulsion plus adjuvants are supposedly responsible, at least according to some investigators, for the encephalomyelitis.

For many reasons the guinea pig offers good material for immunologic, pathologic and early symptomatologic studies. These animals

6. Ferraro, A.: Arch. Neurol. & Psychiat. **52**:443, 1944.

7. Freund, J., and McDermott, K.: Proc. Soc. Exper. Biol. & Med. **49**:548, 1942.

8. Morgan, I. M.: (*a*) J. Bact. **5**:614, 1946; (*b*) J. Exper. Med. **85**:131, 1947.

9. Kabat, E. A.; Wolf, A., and Bezer, A. E.: Science **104**:362, 1946.

10. Ferraro, A., and Cazzullo, C. L.: J. Neuropath. & Exper. Neurol. **7**:3, 1948.

11. Morrison, L. R.: Arch. Neurol. & Psychiat. **58**:391, 1947.

12. Freund, J.; Stern, E. R., and Pisani, T. M.: J. Immunol. **57**:179, 1947.

13. Kopeloff, L. M., and Kopeloff, N.: J. Immunol. **57**:229, 1947.

14. Alvort, E. C., Jr.: Proc. Soc. Exper. Biol. & Med. **67**:4, 1948.

15. Jervis, G., and Koprowsky, H.: J. Neuropath. & Exper. Neurol. **7**:309, 1948.

16. (*a*) Cazzullo, C. L., and Ferraro, A.: J. Neuropath. & Exper. Neurol. **8**:70, 1949. (*b*) Ferraro, A., and Cazzullo, C. L.: ibid. **8**:61, 1949.

17. Kopeloff, L. M., and Kopeloff, N.: J. Immunol. **48**:297, 1946.

are less expensive than monkeys, more easily handled and more easily housed in large numbers, and in them the disease appears more systematically. Moreover, in these animals we are able to produce a diffuse encephalomyelitis with a higher rate of morbidity by employing the intraperitoneal route for the introduction of the antigen.

MATERIALS AND METHODS

The animals, carefully selected, were kept under observation for a period of ten days and then divided into lots of various weights, most of them weighing from 450 to 550 Gm.

In all the experiments we used as an antigen homologous brain taken from ether-killed normal guinea pigs, suspended in 12 per cent isotonic sodium chloride solution in a Waring blender and then incorporated into a water-in-oil emulsion. Falba[18a] and bayol F[18b] were used as emulsifying agents. A given amount of heat-killed tubercle bacilli was dissolved in a small mortar, and a given amount of bayol was then added (table 1). The tubercle bacilli, obtained through the courtesy

Composition of Emulsion

Materials Injected	Amount per Cubic Centimeter	Amount per Guinea Pig	
		Intramuscularly	Intraperitoneally
Normal guinea pig brain.....	0.033 Gm.	3 cc.	1 cc.
Sodium chloride.....	0.4 cc.	.....	.....
Falba [18a]	0.2 cc.	.....	.....
Bayol [18b]	0.4 cc.	.....	.....
Heat-killed tubercle bacilli....	0.33 mg.	.....	.....

of Freund, were of a human type, strain Jamaica 22. After emulsification, the mixture was transferred to sterile bottles, covered with rubber caps and stored in the ice box. A sample of every emulsion was checked for sterility with blood agar plates.

Two lots of guinea pigs were used, numbering 10 and 43 animals, respectively. Each animal of the first lot received an injection of 3 cc. of emulsion into the muscles of the left and right lateral regions of the neck. A 20 gage, 1 inch (2.5 cm.) length needle was used for the injection. Some of the animals temporarily lost their appetite after the injection and decreased in weight within the first twenty-four hours.

Each animal of the second lot received an intraperitoneal injection of 1 cc. of the Freund emulsion. For this injection we used a .22 gage, 1 inch length needle, which was inserted into the lower part of the abdomen along the medial line. The guinea pig was kept in a head downward position by the well known device of putting it, head first, into the examiner's pocket. In our experience, acute peritonitis never occurred.

Many reasons induced us to use the intraperitoneal route for the introduction of the antigen. First, we tried to keep the muscles of the neck free for injections

18. (*a*) Falba® is an absorption base derived from hydrous wool fat and composed of a mixture of oxycholesterol and cholesterol. (*b*) Bayol is a paraffin oil of light viscosity.

of protective substances. Second, we tried to avoid excessive trauma, having received the impression that the intraperitoneal route traumatized the animals very little. Third, we wished to avoid complications due to the slow absorption of the antigen injected intramuscularly. The antigen, which occasionally becomes encapsulated, may be absorbed from time to time, thus influencing the clinical course of the disease. Antigen injected intraperitoneally seems to be absorbed faster as a whole, though one can observe even after long periods a residue of the emulsion in the peritoneal cavity encapsulated in droplike formations of various sizes, some adhering to the various organs.

MORBIDITY AND MORTALITY RATES

The mortality and morbidity following intramuscular injection of the antigen have been reported by others. In the lot of animals of the weight mentioned which received intraperitoneal injections the morbidity was much higher; 39 of the 43 animals contracted encephalomyelitis within the first three weeks after the injection of the antigen.

The mortality was also high. In fact, only 2 of 13 animals of one group survived; 3 of 15 of a second group, and 4 of 15 of a third group. Of the surviving animals of the first group, 1 presented a mild form of the disease; 1 appeared normal. Of the 3 survivors of the second group, 1 suffered from paresis of the posterior limbs and 2 appeared normal. Of the 4 survivors of the third group, 1 was paralyzed, 2 were paretic and 1 may be considered normal. The mortality reached its highest peak within fifteen to twenty-two days.

The appearance of the first symptoms was rapid, and the illness usually followed an acute course. In some cases, following a subacute course, death occurred only after thirty-two to fifty-five days. Some animals not belonging to the series now being reported have lived seventy-eight and seventy-nine days, respectively.

As our experience extends to a larger number of guinea pigs, we feel that a relationship seems to exist between the weight of the animals and the amount of intraperitoneal antigen necessary to precipitate the disease.

SYMPTOMATOLOGIC OBSERVATIONS

Loss of Weight.—The study of the weight curve is particularly interesting. By watching the weight and the general nutrition of the animals, one can somewhat anticipate the onset and progress of the disease, a close correlation of the weight curve and the clinical course having been observed in our animals. There occurs, as a general rule, a drop in weight with the appearance of the first symptoms. Sometimes the loss shortly precedes the appearance of the first symptoms; sometimes it starts simultaneously and progresses steadily until the death of the animals. Particularly interesting is the weight curve of the acute form of the disease, in which the drop occurs earlier and progresses very rapidly to the time of death (fig. 1). On the other hand, in cases in which the clinical course is protracted, the loss of weight appears with the first symptoms and is most impressive during the phase of aggravation. With the stabilization of the disease the animals regain weight and recover good nutrition. In some cases remission and relapses are respectively accompanied or followed by gain and by loss of weight (fig. 2). It is advisable to weigh and examine the animals possibly every day, after feeding.

Related to the weight is the appetite of the animals, which generally decreases shortly before the appearance of the first symptoms. When encephalomyelitis, hemiplegia, ascending paralysis or cerebellovestibular symptoms become fully developed, the animal consumes less food. Convulsive seizures do not seem to affect appetite. Following the period of invasion of the disease, if recovery or improvement sets in, the appetite increases.

Muscular Impairment.—Although it is difficult to make semeiologic observations in animals as small as guinea pigs, it has been possible to observe as first symptoms hypotonia and thinning of the muscles of the lumbosacral region. Asthenia is another early finding, which can best be tested by forcing the animal on its back. The righting reflexes are among the very first to be impaired. In advanced cases of asthenia the animal lies on its back exhausted. The asthenia

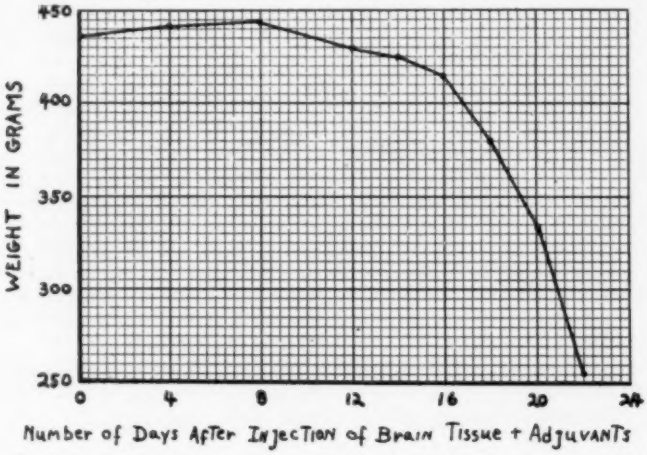

Fig. 1.—Composite weight curve of guinea pigs dying of acute experimental encephalomyelitis.

is followed, or accompanied little by little, by motor involvement related to the localization of the disease. Hypotonia may be present in the absence of asthenia.

Transverse myelitis occurs quite often. In this case the first symptoms are usually hypotonia and thinning of the muscles of the lumbosacral region and of the posterior limbs, deficient righting reflexes and asthenia. Muscular power and motility gradually decrease, and paresis or paralysis of one or two of the posterior extremities sets in, associated with incontinence of feces and urine. Deterioration of the general condition occurs rapidly. Occasionally, the abdomen swells up because of intestinal paralysis, and in certain cases astasia of the trunk and the head and small rhythmic tremors are observed. Trophic changes, such as loss of hair or decubitus, complicate sphincter dysfunctions. Paralysis of the posterior limbs, at first flaccid, later becomes spastic and in extension.

Diffuse encephalomyelitis recalling that of gradual ascending myelitis in human subjects is at times observed, the spinal cord showing the initial invasion and the

involvement progressing rapidly toward the higher centers. In this variety the early symptoms begin also with hypotonia of the muscles of the lumbosacral region, followed by asthenia and paresis, milder in character than the definite flaccid paralysis of the first group. The dysfunction of sphincters appears later and is less pronounced than in the first group. The course of the disease is quite rapid, soon involving the anterior extremities. Here one encounters ataxia and dysmetria and oscillation of major or minor amplitude of the head and trunk. These oscillations may be followed by a rhythmic tremor of the head and the anterior extremities. Subsequently nystagmus occurs, associated at times with symptoms of the cerebellovestibular series.

We never observed paralysis of the tongue or of the muscles of the face, but some difficulty in swallowing was noticed. In the more advanced stage, which is usually reached within a few days, tetraparalysis may develop. Dyspnea sets in and becomes more and more noticeable even at rest, becoming very marked in rest.

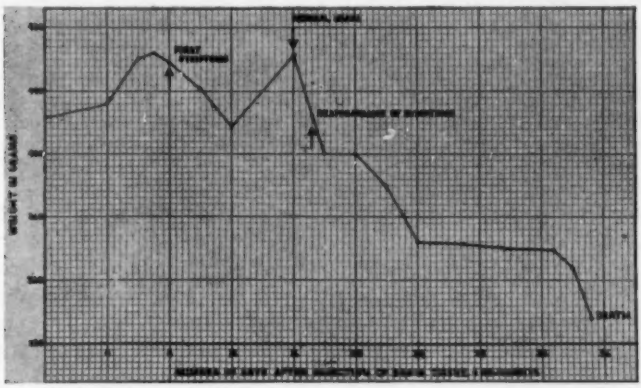

Fig. 2.—Weight curve showing correlation between weight fluctuation and appearance and disappearance of symptoms, relapse and aggravation of experimental encephalomyelitis.

At times generalized convulsions appear. The convulsions usually occur at spaced intervals, but occasionally actual status epilepticus develops. The attacks generally last a few seconds. Some of the attacks are jacksonian in type, becoming generalized later on.

The hemiplegic type is not often encountered. Following the initial general asthenia and hypotonia of the muscles of the lumbosacral region and of the posterior extremities, paresis and then flaccid paralysis of one of the posterior extremities occurs, soon followed by the same occurrence in the homologous anterior extremity. The animal lies on the paralyzed side. Ambulation becomes particularly difficult, but ataxia and tremors are less pronounced. Nystagmus seldom occurs. Localized convulsions, which usually become generalized after some attacks, are at times observed. Transition from hemiparesis to tetraparalysis

is at times encountered, and death usually occurs quite rapidly. The drop in weight is less striking in these cases than in the other two groups.

Of particular interest are the postural changes that occur in a few animals. Some guinea pigs are seen with a twist of head and trunk, so that the back shows a more or less pronounced external convexity, the head and trunk turned more often toward the left. In motion these animals disclose a tendency toward circular walking. The movements are brusque, uncoordinated and at times obviously hypermetric. When the animal is lifted by the trunk and placed again on the ground, it no sooner touches the floor than it resumes its circular posture.

As soon as six or seven days after the intraperitoneal injection, one may note initial neurologic symptoms.

Delayed Paralysis.—In a few instances, following intraperitoneal injection of the antigen, only general asthenia and slight hypotonia of the hindlegs develop. These symptoms may even disappear gradually, although the animal may fail to gain weight. Though the animal is not healthy, no definite neurologic symptoms are manifest. It is only after many weeks or sometimes several months that the clinical picture changes and gradually a marked neurologic syndrome develops, leading to paraplegia of the extremities. Such delayed reactions are under investigation.

Remissions.—While this report was being prepared, interesting observations were made in a group of larger animals. In these animals, remissions were noted, and, following early asthenia, hypotonia and even paresis of one or both extremities, a gradual recovery was observed. The degree of recovery varied from animal to animal.

In these animals showing remission and improvement or even recovery the related histopathologic changes are presently being investigated.

HISTOPATHOLOGIC CHANGES

In the group of guinea pigs in which encephalomyelitis developed following intramuscular injection of brain emulsion plus adjuvants the histopathologic process is to be considered mainly as an acute inflammatory one.

The distribution of the inflammatory reaction was about the same in all the animals. The cerebrum, the cerebellum and the spinal cord were all involved. In the cerebrum, it was the white matter which was mainly affected, the cortex disclosing generally milder changes. However, the soft meninges all over the cerebrum and the cerebellum disclosed an inflammatory reaction, which in certain areas was moderate and followed the folds of the pia-arachnoid in the depth of the cerebral convolutions and in others was intense, with large numbers of inflammatory cells stratified along, or around, blood vessels.

Of the various portions of the cerebrum, the frontal area seemed the less involved. More pronounced was the involvement of the cornu ammonis, especially along the uncus and in the adjacent areas of the hyppocampus, particularly in the subependymal layer of the lateral ventricles.

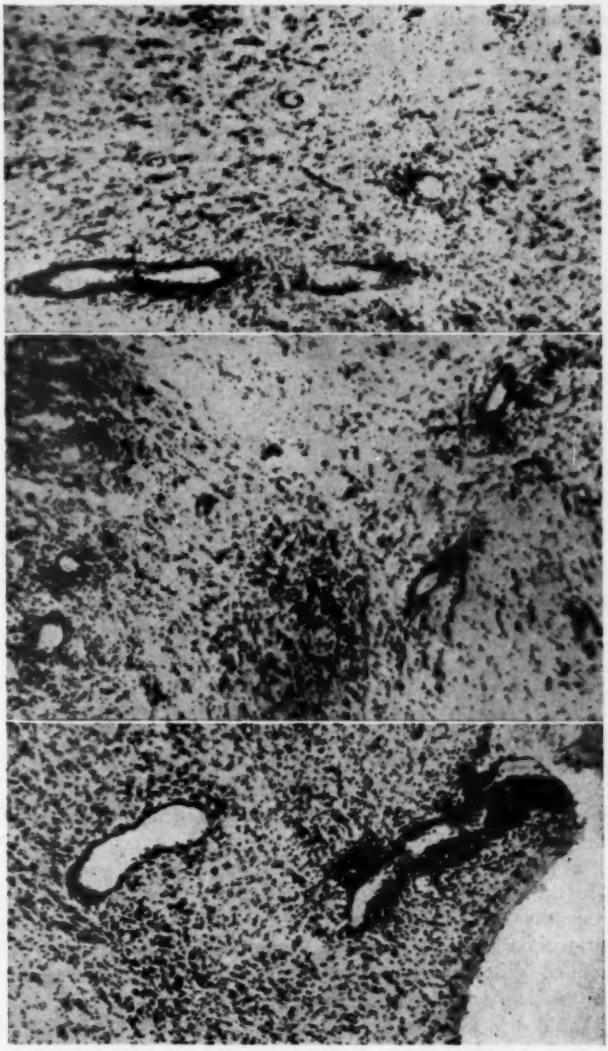

Fig. 3.—Various areas in the pons and the medulla with inflammatory reaction. Nissl stain.

In the brain stem, scanty pathologic change was found in the mesencephalon, although at times the corpora quadrigemina disclosed marked involvement. In the tegmentum of the pons and in the medulla oblongata more intense inflammatory reaction was seen (fig. 3).

In the white matter of the cerebellum patches of inflammatory reaction were often noted, but it was the cerebellar nuclei which constituted an area of predilection for pathologic change.

In the spinal cord the white substance was the seat of the major involvement; the gray matter was involved to a lesser degree. In the meninges, inflammatory cells were often stratified within the pia and along the blood vessels within the septums.

In all the mentioned areas the inflammatory reaction consisted generally in perivascular exudation, the exudate surrounding mostly veins; when the reaction was intense, small and large arteries were also involved.

The exudate consisted of several types of cells: polymorphonuclear elements of the granulocytic series (band cells and segmented cells), lymphocytes, large mononuclear cells, plasmacytes and histiocytes. The polymorphonuclear cells were quite numerous, though not as numerous as the lymphocytes. They were present above all in the areas of more recent involvement and in cases of more recent date. In older lesions the lymphocytes predominated, associated with the large mononuclear elements and the histiocytes. In addition to the histiocytes originating from blood vessels there were histiocytes in the formation of which the microglia and oligodendroglia cells had participated.

In our material the plasmacytes were not as numerous as reported by others. Only here and there were we able to detect them with the Nissl stain. It is possible, however, that with more specific stains for plasmacytes one might detect a larger number of them.

Large mononuclear cells were more commonly encountered in cases in which the animals had survived long periods.

The major contribution to the histiocytic reaction was furnished by the microglia cells. These cells were seen even with the Nissl stain as participating in the exudation, partly mixed with other cells and partly surrounding the layer of lymphocytes (fig. 4). Specific impregnation with the Hortega method has not been very successful in our material and only fragments of the histologic picture are available. Compound granule cells derived from microglia cells seemed less numerous than large mononuclear cells.

We failed to find definite correlation between the age of the pathologic process and the type of the perivascular exudate. This is due in our opinion to the fact that in the course of a long-standing process, leading to chronic lesions, new showers of the cells characterizing the acute reaction occur with renewed output of antigen from the site of

injection, as a result of which the process is often a mixed one: acute, subacute and chronic. The life span of the animals surviving outstanding neurologic symptoms has not been long, and further investigations are necessary to solve this problem.

In 2 animals which survived the onset of the paralysis several weeks, we found that the inflammatory reaction involving both brain and spinal cord was mild in one and severe and diffuse in the other.

The same comment applies to the reaction of the glia cells. We refer particularly to the astrocytes, which in the early stages disclosed acute regressive changes in the midst or in the immediate vicinity of

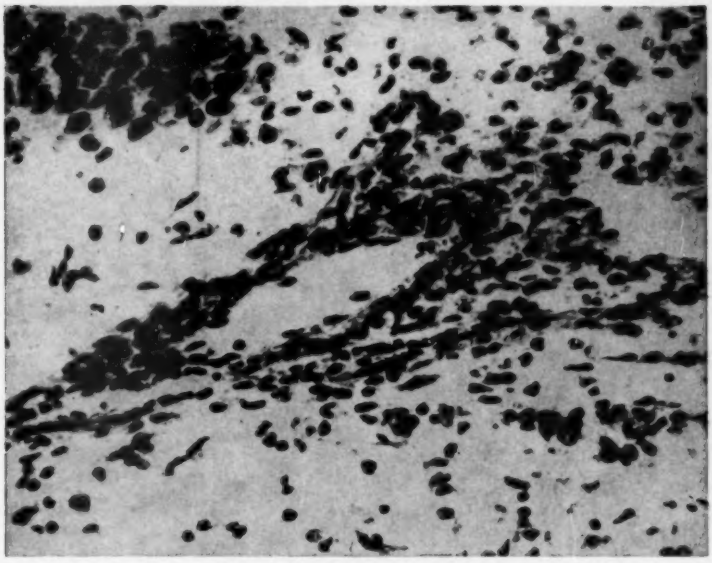

Fig. 4.—Microglial reactions surrounding a blood vessel. Lymphocytes are intermingled with these cells. Nissl stain.

an inflammatory area. Later on, a distinct type of progressive reaction occurred. It consisted of hypertrophy of astrocytes, visible especially in animals which had survived several weeks with neurologic symptoms (fig. 5). In the spinal cord, where often the inflammatory lesions were more pronounced, one failed to observe such a progressive reaction. This applies particularly to the gray matter where one got the impression that irrespective of the duration of the disease a regressive change of the astrocytes, clasmatondendrosis, was prevalent. In the white substance a better attempt at progressive reaction was noticeable,

never, however, reaching the intensity detected in the cerebral white matter.

The reaction of the blood vessels in the involved area varied from case to case. Any relationship existing between the reaction of the blood vessel walls and the severity of the inflammatory process was not definite. Numerous blood vessels disclosed swelling of the endothelial lining cells. Some disclosed a thickening of all layers of the wall, with predominance at times in the adventitia, the media or the intima. Often one noticed a deformity of the intima resulting from

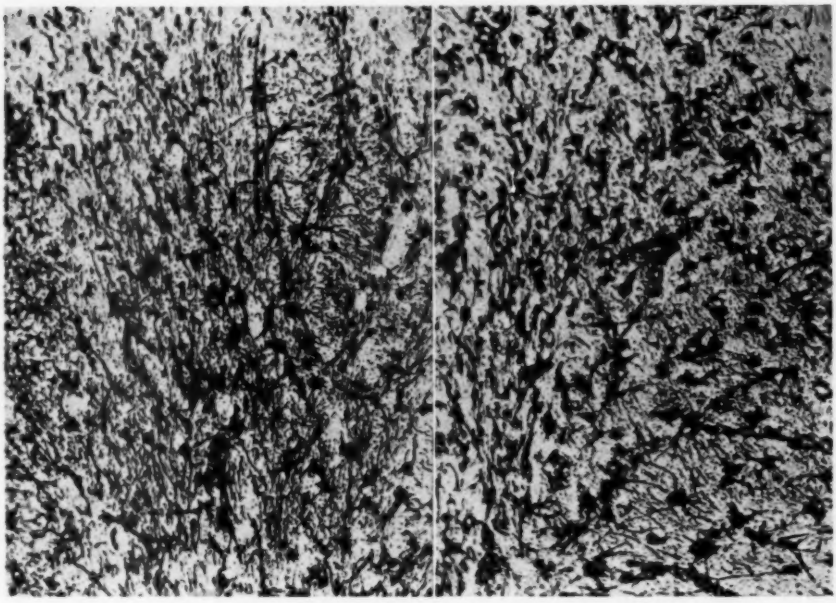

Fig. 5.—Progressive changes of astrocytes (hypertrophy and hyperplasia) in the white substance. Cajal gold chloride-mercuric chloride method.

unequal thickening and folding of the intimal layer. At times the thickening of the intima and the intramural and perivascular reaction was of such intensity that partial or total occlusion of the blood vessel lumen occurred. No fresh thrombi were detected with the Nissl or the hematoxylin-eosin stain.

Myelin sheath and axis-cylinder preparations disclosed a moderate amount of change. Generally speaking, we have failed to find the typical areas of demyelination reported occurring in monkeys in the same type of encephalomyelitis. Here and there in spreading areas

of inflammatory reaction only rarefaction and occasionally destruction of myelin sheaths were visible.

The axis-cylinders, particularly those of the spinal cord, disclosed, in relation with a more severe type of inflammatory reaction, various degrees of swelling and fragmentation.

In the group of guinea pigs in which encephalomyelitis followed intraperitoneal injection of brain emulsion plus adjuvants, the fundamental pathologic process in both brain and spinal cord was substantially identical with the one occurring in animals whose encephalomyelitis followed intramuscular injections.

In the brain, particularly, most of the pathologic features were identical as to type, distribution and intensity of the process. (*a*) Type: A diffuse meningeal reaction and perivascular reaction extended to numerous areas of the brain, with predilection for the white substance. The meningeal exudate, especially in the later stages, was mainly formed by lymphocytes, large mononuclear cells and numerous chromatophores. There the perivascular exudate was also predominantly formed of lymphocytes and large mononuclear cells. In less advanced stages, in guinea pigs which survived only a few days after the onset of the first neurologic symptoms, polymorphonuclear cells were also present and plasmacytes were occasionally found. As with the intramuscular way, no giant cells were detected.

(*b*) Distribution: The distribution of the perivascular reaction followed in this group the same pattern reported in the first group, i. e., predilection for the periventricular areas, the diencephalon, the medulla and particularly the vestibular nuclei and the nuclei of the cerebellum proper.

(*c*) Intensity: The intensity of the pathologic reaction varied from case to case, bearing no strict relationship to the length of survival of the animals.

In the spinal cord, however, there seemed to be some variants of the changes resulting from the intramuscular injections. The difference was in both intensity and type of perivascular reaction. We feel that the process in the spinal cord was more severe following intraperitoneal injection of antigen. Most of the white columns of the spinal cord appeared severely involved. In one case severe involvement was present after a survival of fifty-two days whereas in another the same severe involvement was present only seven days after the onset of the first symptoms.

Not only a quantitative but also a qualitative difference is appreciated in the sense that surrounding the blood vessels mostly lymphocytes and large mononuclear cells were seen. The number of inflammatory cells and their compactness were such that the involved

blood vessels recalled the appearance of periarteritis nodosa. Though no necrotic changes were seen, higher power magnification brought out more clearly the abnormal thickening of the blood vessel walls,

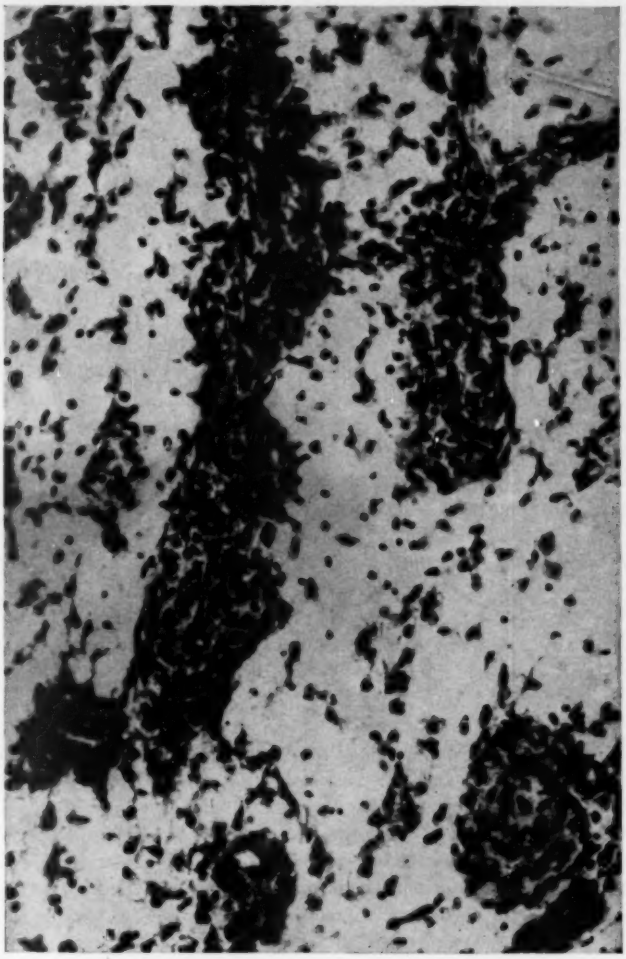

Fig. 6.—Severe perivascular reaction in lateral columns. Nissl stain.

leading in certain instances to complete mechanical occlusion of the blood vessel's lumen (fig. 6).

Myelin sheath stains pointed to generally well preserved myelin sheath in both cerebrum and cerebellum. The glia reaction did not differ from that resulting from intramuscular injection of antigen. The same applies to the axis-cylinders.

In the two groups in which encephalomyelitis resulted from intramuscular and intraperitoneal injections of antigen, respectively, hemorrhages and necrosis were not outstanding. Nothing comparable to the Arthus phenomenon has ever been observed in our material. Occasional necrosis has been detected in the white matter of the spinal cord and only occasional fibrinoid necrosis of the blood vessel walls.

Concerning the reaction of other parenchymas, lung, liver, spleen, etc., we have drawn from our material interesting pathologic data contrasting with the negative results of other investigators. A detailed report will form the object of a separate communication.

Relationship of the Encephalomyelitis Produced in Guinea Pigs and That Produced in Monkeys.—It is important to raise this question in view of the fact that in guinea pigs the demyelination was not an essential part of the histopathologic process. Because of this variation, some investigators might feel that the two processes are different. Our contention is that the encephalomyelitis of guinea pigs produced with the same antigen used for monkeys and as a result of the same pathogenic mechanism is identical with the encephalomyelitis experimentally induced in monkeys.

Not all animals react in the same manner to the same etiopathogenetic factors. Dogs react with endarteritis proliferans to lead poisoning—cats do not. Dogs react with softening of the globus pallidus to carbon monoxide poisoning—rabbits do not. Let us not forget that next to the chapter of general pathology there is the chapter of comparative pathology, which is still to be fully investigated in animals.

It is important to establish the identity of the process in both guinea pigs and monkeys because of the fact that in the progress of our investigation we need animals that are easily handled, relatively inexpensive and easily housed in large numbers. In our further immunologic studies and studies to assay various methods for the prevention of the disease, we want to feel that we are dealing in guinea pigs with the same fundamental pathologic process which in monkeys is associated with demyelination.

SUMMARY

Emulsion of 3 cc. of normal brain plus adjuvants, according to Freund's technic, introduced intramuscularly produces diffuse encephalomyelitis in guinea pigs; 1 cc. of the same emulsion introduced intraperitoneally produces the same type of encephalomyelitis. The

reasons for using this route are discussed. Symptomatologically, the disease involves the whole nervous system. Initial symptoms are hypotonia and asthenia, evidenced by poor righting reflexes. Ataxia, paresis, paralysis, tremors and convulsions frequently develop. The paralysis of the posterior limbs is flaccid, becoming spastic later on. The lesions are diffuse, but one can find a sort of predilection for the spinal cord, the brain stem and the cerebellum. Variations in the weight and the nutrition of the animals are discussed. Histologically, the main feature is a perivascular inflammatory reaction of the brain and spinal cord, an expression of allergic encephalomyelitis. Demyelination is scarce whereas progressive glia reaction is encountered. Hemorrhages and necrosis are scanty. The clinical and the pathologic features of this encephalomyelitis bear close similarities to the encephalomyelitis induced by the same procedure in monkeys.

SKELETAL GROWTH AND DEVELOPMENT IN MICE FED A HIGH PROTEIN DIET

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ST. LOUIS

THE NUTRITIONAL requirements for growth and maintenance have been extensively studied by withdrawing certain constituents from the diet. However, less attention has been paid to the effect of feeding excessive amounts of such basic components as fat, protein or carbohydrate. Data concerning weights and chemical analyses of the carcass are available, whereas reports of histologic changes in the skeleton are lacking.

In continuation of our investigations of the role of nutritional factors in skeletal growth and aging we have analyzed the course of these processes in young mice fed a diet high in casein. The results obtained will presently be described.

MATERIAL AND METHODS

Fifty-two virgin female mice of the inbred strain C57 black raised in our laboratory were used. At the age of 4 weeks, the animals, weighing then about 10 Gm., were divided into two groups:

Series 1.—Twenty-six mice were fed a stock diet of a commercial chow [1] which contains the following ingredients:

	Per Cent
Moisture	8.90
Protein	26.18
Fat	5.35
Fiber	4.62
Ash	6.49
Nitrogen-free extract	48.46
Calcium	1.17
Phosphorus	0.87

From the Snodgras Laboratory of Pathology, City Hospital, and the Department of Pathology, Washington University School of Medicine.

This investigation was supported by the American Cancer Society on recommendation of the Committee on Growth of the National Research Council and by a grant from the Committee on Scientific Research of the American Medical Association.

1. The chow used was B-2362 made by the Ralston Purina Company, St. Louis. Data concerning the mineral and vitamin contents can be found in the pamphlet issued by the company.

Series 2.—Twenty-six mice received a diet high in casein and of the following composition:

	Per Cent
Crude casein	52.69
Crude fat	2.64
Crude fiber	2.32
Nitrogen-free extract	29.49
Ash	3.96
Calcium	1.24
Phosphorus	0.70

An adequate vitamin content was assured by the use of 4 per cent brewers' dried yeast, 4 per cent dehydrated alfalfa meal and a special vitamin premix containing vitamin D, riboflavin, thiamine and nicotinic acid.[2]

This ration was given to the animals as a finely ground meal in unlimited amounts and was readily eaten. Water was likewise given ad libitum. Usually

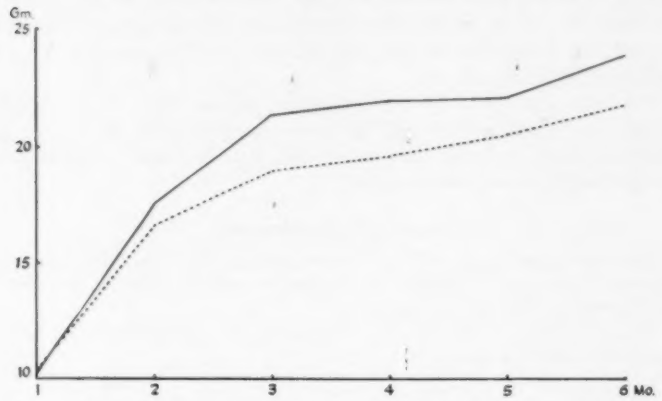


Fig. 1.—Mean weight curves of mice fed the stock ration (———) and mice fed the high casein ration (.....).

6 animals were kept in one enamel pan measuring 12½ by 8½ by 4½ inches (about 32 by 21.5 by 11.5 cm.). Weights were taken once a week up to the age of 4 months; thereafter the mice were weighed at monthly intervals. The animals were killed in the following order: Four mice of each group at the ages of 6 weeks or 2 months, and 6 mice of each group at the ages of 3, 4 or 6 months, respectively.

At necropsy the individual weights were recorded; the tibia, the femur and the knee joint were removed as a whole; vertebrae and pieces of internal and endocrine organs were secured and fixed in 4 per cent formaldehyde solution. The bones were decalcified in 5 per cent nitric acid, neutralized in 5 per cent alum (aluminum and potassium sulfate) and embedded in paraffin. Semiserial sections were prepared and stained with hematoxylin and eosin for microscopic examination.

2. The Ralston Purina Company cooperated in the preparation of this ration.

EFFECT ON WEIGHT

As seen from figure 1, the animals kept on the high casein diet gained weight steadily but did so more slowly than those receiving the stock ration.

The table shows the mean weights and the maximum and minimum deviations.

The individual weights of the mice fed the stock diet showed considerable variation; however, there were but slight differences in the individual weights of the animals receiving the high protein ration.

HISTOLOGIC OBSERVATIONS

The description of the tissue changes is based on observation of the growth zone at the upper end of the tibia.

Series 1: Animals 6 Weeks Old.—In mice fed the stock ration, the growth zones were about 200 microns wide (fig. 2*A*). The cartilage cell rows showed

The Mean Weights (in Grams) and the Maximum and Minimum Deviations

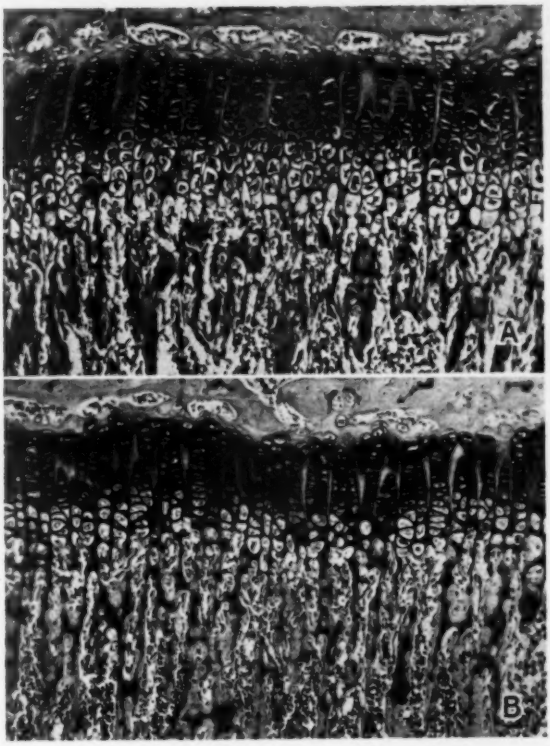
Age of Animals, Mo.	Animals Fed the Stock Diet		Animals Fed the High Casein Diet	
1 (initial)	10.2	maximum 0.8 minimum 0.2	10.4	maximum 1.1 minimum 0.4
2	17.5	maximum 0.5 minimum 2.5	16.6	maximum 0.4 minimum 0.6
3	21.3	maximum 1.7 minimum 1.3	18.9	maximum 0.1 minimum 0.9
4	21.9	maximum 2.1 minimum 1.9	19.6	maximum 0.4 minimum 0.6
5	22.1	maximum 1.9 minimum 2.1	20.5	maximum 0.5 minimum 0.5
6	23.9	maximum 2.1 minimum 2.9	21.8	maximum 0.2 minimum 0.8

regular configuration and were composed of 8 to 10 small columnar and 3 or 4 hypertrophic cells. Here and there, mitotic figures were seen in the upper layers of the cartilage columns. Thin layers of chondromucoid ground substance separated the individual cartilage cell rows from one another. The metaphysis was vascular; the primary spongiosa was represented by thin, short trabeculae. The shaft was composed of large osteocytes and abundant interstitial substance. The articular cartilage contained two layers of small spindle-shaped cells in the sliding zone; the resting cartilage cells were being converted into proliferating and hypertrophic cells.

In mice fed the high casein diet for two weeks, the growth zones measured an average of 140 microns (fig. 2*B*). The cartilage showed the regular structure and increased calcification of the matrix. In the individual cartilage cell row 6 to 8 columnar and 2 or 3 hypertrophic cells were counted. Active growth was indicated by the numerous mitotic figures appearing in the columnar cartilage cells. The latter were larger, and the breakdown of the hypertrophic cartilage cells occurred farther proximally than ordinarily. The primary spicules were more numerous, thicker and longer than in the animals receiving the stock ration. The

osteocytes of the shaft were small, and the bony ground substance was markedly calcified. The articular cartilage was represented by fewer undifferentiated and more hypertrophic cells, and bone replacement of cartilage was farther advanced than in the mice kept on the stock diet.

Series 2: Animals 2 Months Old.—In mice receiving the stock diet, the growth zones were about 125 microns wide (fig. 3*A*). The cartilaginous matrix was more



Figures 2 to 5 show sections through the epiphysial growth zones at the upper ends of tibias of virgin female mice of strain C57 black; × 112.

Fig. 2.—*A*, 6 week old mouse fed the stock diet. The growth zone and the metaphysis show the usual structure.

B, 6 week old mouse fed the high casein diet for two weeks. As compared with *A* the growth zone is narrowed, the columnar cartilage cells are larger, and there is more bone in the metaphysis.

abundant than at the age of 6 weeks, and small wedges of hyaline material were noted between the cartilage cell rows. The latter were composed of 5 to 8 columnar and 2 to 3 hypertrophic cells. The metaphysial trabeculae were slender

and short and covered by continuous layers of large osteoblasts. The appearance of the shaft and the articular tissues was similar to that noted at the age of 6 weeks.

In mice kept on the high casein diet for 1 month, the growth zones were intensely calcified and varied in width from 125 to 140 microns (fig. 3*B*). The individual cartilage cell rows contained 5 to 7 large columnar and, at best, one

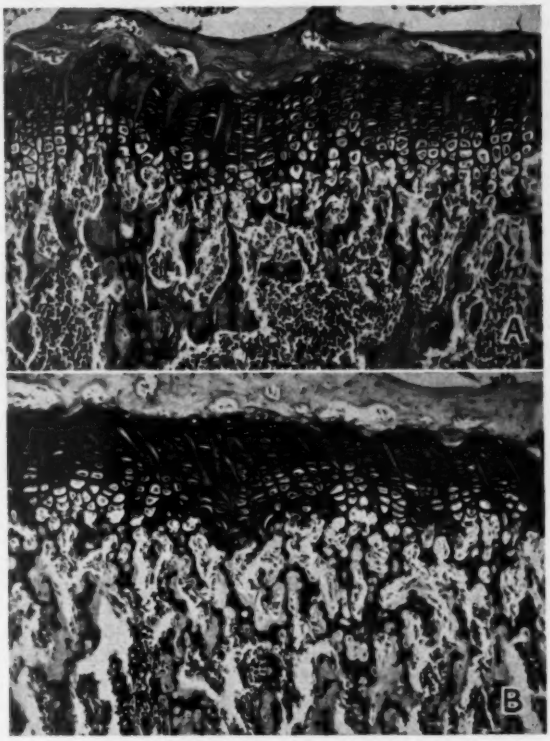


Fig. 3.—*A*, 2 month old mouse fed the stock diet. The cartilaginous matrix is more abundant and the trabeculae are shorter and more compact than in the younger animal (fig. 2*A*).

B, 2 month old mouse fed the high casein diet for one month. The growth zone is irregular in width; the columnar cartilage cells are larger, the hypertrophic cells are fewer, and the metaphysial spicules are longer and thicker than those of the control animal (fig. 3*A*).

fully developed hypertrophic cell. Again, the columnar cells showed many mitotic figures and began to enlarge in the more proximal parts of the growth zones. In the metaphysis were numerous short, broad-based spicules, some forming transverse bridges. The matrix of the shaft contained thick, distinct calcium lines. The

articular cartilage was not remarkable. Much subchondral bone had been laid down.

Series 3: Animals 3 Months Old.—In the mice receiving the stock diet, the average width of the growth zones was 75 microns. The cartilage cell columns could still be recognized; however, large wedges of dense hyaline ground substance separated the cartilage cell rows from one another or began to replace them. The cartilage columns were shorter than at the earlier ages and composed of 5 to 7 columnar and 2 or 3 hypertrophic cells. The vascularization of the metaphysis was decreased; the spicules contained larger amounts of calcium; they were short, covered by small osteoblasts and connected with one another by bony links. The shaft was composed of small osteocytes and heavily calcified matrix. The articular tissues had not changed as compared with those of the younger age group.

In mice fed the high casein diet for two months, the growth zones were about 80 microns wide. The cartilaginous ground substance had slightly increased in amount. The individual cartilage cell rows were composed of 6 to 8 columnar and 2 or 3 hypertrophic cells. The cartilage cells proliferated actively, as was indicated by the presence of many mitotic figures. The hypertrophy of the columnar cells, however, was now less conspicuous than at the earlier ages; these cells enlarged only after they had come to lie near the distal end of the cell rows. Fully developed hypertrophic cells were more numerous than before. The vascularization of the metaphysis was good; many spicules were seen in the subchondral layer. The cortex of the shaft and the articular tissues were not remarkable.

Series 4: Animals 4 Months Old.—In mice fed the stock ration, the growth zones were narrow and had become irregular in width (fig. 4*A*). Much hyalinized intercellular substance was present. The cartilage cell rows contained 4 or 5 columnar and 1 or 2 small cells of hypertrophic type. The proliferation of the columnar cartilage cells was at a low or had come to a standstill. Single cartilage cells or whole cartilage cell rows had broken down, and plugs of amorphous material had taken the place of destroyed cartilage. These plugs were thick and frequently showed horizontal cracks. While some bony trabeculae were still present, the epiphysial cartilage was separated from the metaphysis by a continuous thick transverse osseous lamella. The cortex of the shaft had increased in thickness. The articular cartilage was in a resting condition; the ligaments and the synovialis were not unusual.

In mice kept on the high casein diet for three months (fig. 4*B*), the width of the growth zones was about 80 microns. The individual cartilage cell rows were composed of 5 columnar and 2 fully developed hypertrophic cells. Here and there mitotic proliferation of the columnar cells was observed. The hyalinization of the matrix and the regressive alteration of the cartilage cells were less advanced than in the animals fed the stock ration. Metaphysial trabeculae were numerous and showed horizontal bridges. In only a few instances a thin and discontinuous bony lamella was seen underneath the epiphysial cartilage. The compacta of the shaft was not remarkable. The articular cartilage cells were large. In 2 of the 6 animals of this group the cartilage of the intermediate zone had undergone hypertrophy; in addition, a number of cell nuclei showed pyknosis or karyolysis. These degenerating cells were surrounded by a rim of basophilic matrix. In 2 other animals the collagenous tissue of the ligaments was loosened and had undergone mucoid change.

Series 5: Animals 6 Months Old.—In mice fed the stock diet, the zones of growth consisted of a narrow plate of inactive, sclerosed and hyalinized cartilage

(fig. 5*A*). Numerous cartilage cell rows had been destroyed, and many thick, amorphous, partly calcified or ossified plugs traversed the epiphysial disks. There was no longer any difference between the cells of columnar and hypertrophic type. The inactive epiphysial plate was sealed off from the metaphysis by a thick continuous bony lamella, indicating that epiphysial growth had ceased altogether. Here and there, a bony spicule was seen in the metaphysis. There was no indication

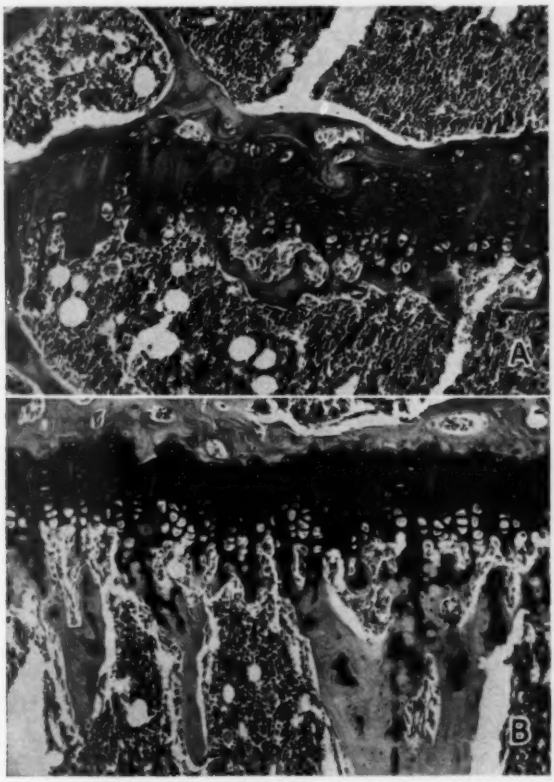


Fig. 4.—*A*, 4 month old mouse kept on the stock diet. There is marked hyalinization of the cartilage; a transverse bony plate is forming underneath the cartilage; most of the primary spongiosa has been resorbed.

B, 4 month old mouse fed the high casein diet for three months. The columnar arrangement of the epiphysial cartilage is better preserved and hyalinization is less advanced than in the control animal (*A*). The primary spongiosa is still present and represented by thick, long trabeculae; the transverse osseous plate, seen in *A*, has not yet formed.

of an actual or impending break-through suggestive of beginning epiphysiodiaphysial union. The cortical bone was thick and dense. The articular cartilage was

inactive except in 1 animal in which a slight hypertrophy of the cells was noticeable. Neither the synovialis nor the ligaments showed pathologic change.

In mice fed the high casein diet for five months, the growth zones were represented by cartilaginous plates showing but little activity and composed of rows of 4 or 5 columnar cells and 1 or 2 small cells of hypertrophic type (fig. 5*B*). The amorphous plugs of degenerated cartilage were more numerous than at the earlier age but not always quite as large and cracked as in the control animals.

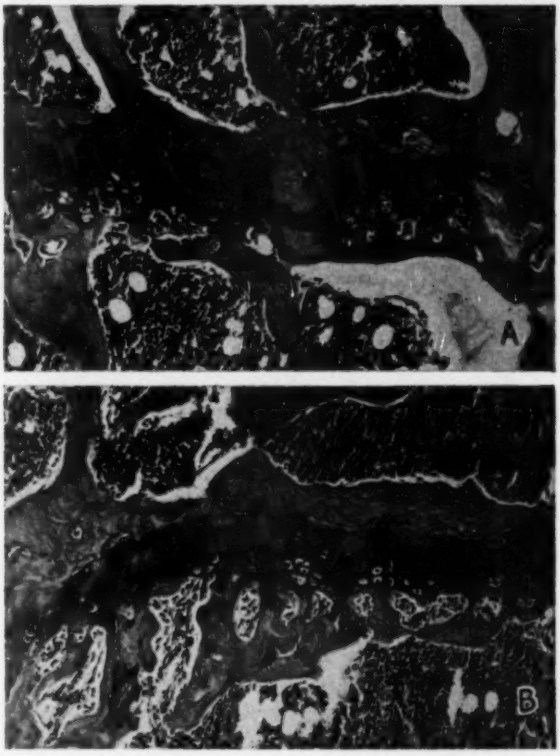


Fig. 5.—*A*, 6 month old mouse fed the stock diet. Growth of cartilage has ceased; some bone replacement of cartilage is still going on (left side of photograph). Many cell rows have been replaced by plugs of amorphous material, which show transverse cracks. To the right the cartilage is sealed off from the diaphysial cavity by a solid lamella of bone.

B, 6 month old mouse fed the high casein diet for five months. The structure of the growth zone begins to resemble that of the control animal (*A*). However, the plugs of degenerated cartilage are not as well defined as in the latter, bone replacement of cartilage is in progress everywhere, and the cartilage has not yet been sealed off from the diaphysial bone marrow.

The subchondral osseous plate was discontinuous, and bone replacement of cartilage was still in progress. The cortex of the shaft contained small and

dense osteocytes. Of the 6 mice of this series, 1 showed moderate hyperplasia and hypertrophy of the articular cartilage, and 2 others swelling and fibrinoid change of the ligaments (fig. 6*A*). The synovialis was hyperplastic and penetrated the articular surface, following enlarged preformed vascular channels, causing focal demineralization of bone and replacement of some of the epiphysial marrow (fig. 6*B*).

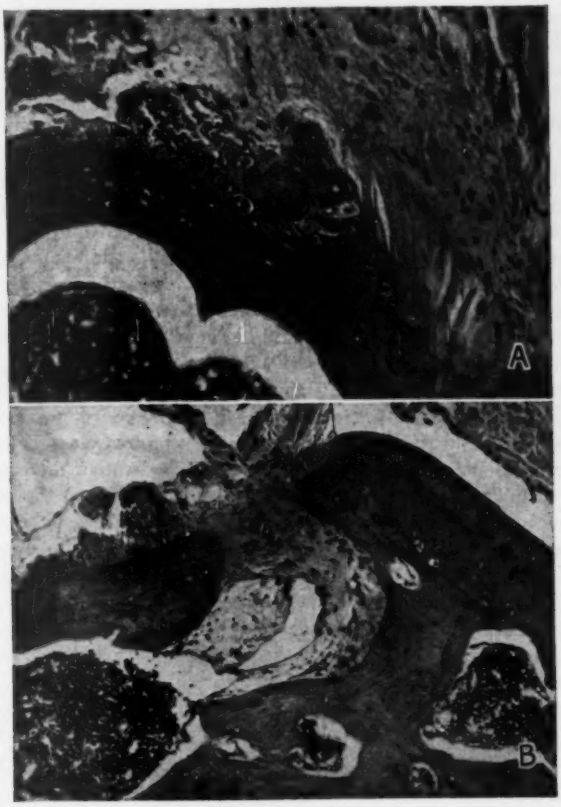


Fig. 6.—*A* and *B* represent sections through the tibial surfaces of knee joints of 6 month old virgin female mice of strain C57 black kept on the high casein diet for five months. × 200. In *A* there is an area of fibrinoid change in one of the cruciate ligaments near its insertion. In *B* synovial tissue penetrates the articular surface and replaces some of the epiphysial bone marrow.

COMMENT

In virgin female mice of strain C57 black fed a stock diet containing 26.18 per cent protein, the growth of the tibia and the femur pro-

ceeded until the age of 4 months. From the third month of life on, regressive processes developing in the epiphysial cartilage increased until they outbalanced those of growth, a state characteristic of the second phase of the skeletal time curve.[3]

In virgin females of the same strain fed a high casein diet (52.69 per cent) the development of the epiphysial cartilage was accelerated and bone formation intensified during the early period of epiphysial growth. These findings are in agreement with observations of accelerated body growth of rats receiving a high protein diet.[4] However, in our mice the onset and progress of regressive changes in the epiphysial cartilage were not hastened in proportion to the intensification of the growth processes: At 4 months of age the growth zones of mice reared on the high casein diet appeared more youthful than those of the animals receiving the stock ration. At the age of 6 months, when the present experiments were terminated, the differences between the control and the experimental groups were less conspicuous, although in some animals receiving the high casein ration the cartilage was still better preserved than in the control series. Whether or not this initial delay of degenerative processes might, at a later age, be compensated by an acceleration and intensification of age changes will have to be decided by long range experiments. Enhanced skeletal aging following initial retardation has been observed in mice and guinea pigs subsequent to ovariectomy.[5]

The mechanism through which the dietary protein acts on the cartilage is unknown. In rats fed a protein-enriched ration there was an absolute and relative decrease of the calcium content of the carcass. This loss could be prevented by adding calcium to the diet.[5] The calcium requirements of the mouse are relatively low, and the average diet usually contains more than adequate amounts of this mineral. It is thus difficult to produce a calcium deficiency in mice. Still, the slower aging of the epiphysial cartilage might be correlated with decreased deposition of lime salts, although there was no histologic evidence of generalized demineralization of the bone itself.

While excessive dietary casein supplied during the growth period may be utilized for skeletal growth and development, this protein did not exert an early injurious effect on the epiphysial and articular cartilages as did excessive amounts of dietary fat.[6] In animals fed the high casein diet the articular tissues showed but small foci of hyperplastic cartilage, an occasional degenerated cartilage cell, localized fibrinoid change in a ligament and a slight mucoid degeneration or proliferation of the syno-

3. Silberberg, M., and Silberberg, R.: Arch. Path. **36**:512, 1943.

4. Sherman, H. C., and Pearson, C. S.: Proc. Nat. Acad. Sc. **33**:264, 1947.

5. Sherman, H. C.; Ragan, M. S., and Bal, M. E.: Proc. Nat. Acad. Sc. **33**:356, 1947.

6. Silberberg, R., and Silberberg, M.: Am. J. Path., to be published.

vialis. In particular, the changes in ligaments and synovialis were found without or with negligible involvement of the cartilage. Conversely, in mice fed the high fat diet the regressive changes predominated: There were advanced degeneration, hyperplasia and hypertrophy of the articular tissues, and the synovial reaction occurred in association with severe alterations of the cartilage. Whether or not the slight synovial changes found after casein feeding will become intensified after prolonged ingestion of the high protein ration, and whether or not additional changes will develop in the articular tissues under these conditions, remains to be seen. Possibly several types of degenerative lesions of joints may occur in the mouse differing in pathogenesis but leading, in the end, to more or less uniform morphologic manifestations.

The role of sex in the skeletal response to nutritional factors has yet to be considered. Sex differences were found in the body growth of rats receiving a high casein diet, the males gaining weight more rapidly than the females.[4] Since the male is genetically larger than the female, excessive protein may be better utilized by the male in the building up of the skeleton. Male mice fed a high casein diet (unpublished data) likewise gained weight more rapidly than females, whose weights in spite of good skeletal growth remained about 10 per cent below those of their controls. The early degenerative lesions of joints following the feeding of a high fat diet were noted in male mice. The lack of more severe articular changes in the females kept on the high casein ration may be partly attributable to the influence of hormonal factors: In females the regular cyclic output of estrogenic hormones increases the density of the cartilage and thus renders it less vulnerable to injurious influences. Investigations are in progress to test this assumption.

SUMMARY

In growing female mice of strain C57 black, a diet containing 52.69 per cent casein accelerated the growth and development of the epiphysial cartilage and increased bone formation. The onset and progress of the regressive processes that occur in the cartilage were temporarily delayed. At the age of 6 months the age changes observed in the skeletons of mice fed the high casein ration were of about the same order as those seen in mice kept on the stock diet. Only minor degenerative alterations of the articular tissues and slight proliferation of the synovialis occurred under the influence of the high casein diet.

THE HUMAN AORTA

Sulfate-Containing Polyuronides and the Deposition of Cholesterol

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THE CONDITIONS under which cholesterol is deposited in the human aorta and in tissues attacked by hereditary xanthomatosis have been studied in previous papers.[1] It was shown that besides the cholesterol available from the serum there must exist a tissue factor which is of importance in determining the localization of the deposits.

Cholesterol is present in the body as a necessary constituent of all cells. Larger amounts are found in certain cellular systems, in liver and in nerve tissue. This cholesterol, however, is intracellular and will follow the rules for the individual cell type.

In addition there exists, scattered through the organism, a series of cholesterol deposits which have such features in common that a joint treatment should be justified. This cholesterol is mainly extracellular. If intracellular, it is found in foam cells, indicating that the deposition was primarily extracellular. These deposits all increase with advancing age, and occur earlier when the cholesterol content of the serum is increased. Table 1 presents a survey of these deposits.

As shown in table 1, these tissues have another common feature. All show metachromasia when treated with toluidine blue, and this metachromasia is ethanol resistant. The metachromasia can be present normally (cornea, cartilage, aorta) or as a result of morbid changes (inflammatory conditions, experimental atheromatosis). This metachromasia must, according to Lison,[2] be assumed to show the presence of polymerized carbohydrate–sulfuric acid esters: heparin, chondroitin–sulfuric acid, hyaluronic acid–sulfate. From several of the tissues mentioned one of these substances has been isolated; in others they have been shown by histologic studies only.

Cholesterol deposits of this type are found only where ethanol-resistant metachromasia is present, and, conversely, where this metachromasia is present, such a deposition is to be expected. Hence it is

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This study was aided by a grant from the P. Carl Petersen Foundation.

From the Copenhagen County Hospital Medical Department F and the Health Insurance Physicians Laboratory, Copenhagen, Denmark.

1. Faber, M.: (*a*) Acta med. Scandinav. **124**:545, 1946; (*b*) **125**:210, 1946.
2. Lison, L.: Histochimie animale, Paris, Gauthier-Villars, 1936.

justified to propose as a working hypothesis that the presence of ethanol-resistant metachromasia will be the histologic evidence for the

TABLE 1.—*Cholesterol Deposits*

Localization	Serum Cholesterol Level During the Deposition	Metachromasia or Known Ester Sulfate	Comments on the Nature of the Deposition of Lipid
Normal Changes in Man			
Cartilage	Normal	Chondroitin-sulfuric acid (Mörner, K.: Skandinav. Arch. f. Physiol. 1:210, 1889)	Rising amounts of cholesterol with age,[6] at first below the perichondrium, later also around the enchondral vascularization (Schultz, cited by Bürger and Schlomka [4])
Arcus senilis corneae	Normal and increased	Hyaluronic acid sulfate (Meyer, K., and Chaffee, E.: Am. J. Ophth. 23:1330, 1940)	Increasing clinical frequency with age; occurs earlier in hypercholesteremia [1b]
Normal aorta	Normal and increased	Chondroitin-sulfuric acid [9]	Increasing with age; occurs earlier and is more pronounced in hypercholesteremia [1b]
Pathologic Changes in Man			
Hypertensive aorta	Normal and increased	Chondroitin-sulfuric acid	More pronounced depositions than in the normal aorta (Björnsson, J.: Arteriosclerosis: A Chemical and Statistical Study, Copenhagen, E. Munksgaard, 1941
Inflammatory tissues: Acute	Increased	Metachromasia Heparin? (Sylven, B.: Acta chir. Scandinav. [supp.] 66:1, 1941)	Xanthomas in healing herpes zoster lesions
Chronic	Normal	Metachromasia Heparin? (Sylven—cited above)	
Chronic parametritis	Normal	Metachromasia (Sylven—cited above)	Frequently considerable amounts of foam cells [5]
Cholesterol pleurisy	Normal	.....	Large amounts of cholesterol in chronic pleural effusions
Syphilitic aortitis	Normal	.....	The aortas with the highest cholesterol content (Björnsson—cited above)
Hand-Schüller-Christian disease	Normal and increased	?	Not known whether metachromasia is found in this tissue, but highly probable
Experimental Lesions			
Atheromatosis	Increased	All experimentally produced forms of atheromatosis are accompanied by reparative changes. In several forms metachromasia has been demonstrated (Erb [12]; Ssolowjew [8])	
Arcus senilis in rabbits	Increased	Metachromasia	

presence of the tissue factor and that these carbohydrates themselves may be the tissue factor.

It is, however, known that polyuronides of this type can be isolated from tissues which do not show ethanol-resistant metachromasia. In these tissues, there is reason to believe, the polyuronides are bound

more firmly to the proteins, as shown by the fact that their extraction free of protein is more difficult. This more firm binding may also inhibit the effect of the polyuronides on the deposition of cholesterol. This will, for instance, be the case of cutaneous tissue, which contains chondroitin–sulfuric acid [3] but which does not give ethanol-stable metachromasia.

As mentioned, the ethanol-stable metachromasia can be a normal finding in an organ. In this case a deposition of cholesterol will be present in all human beings above a certain age limit, as has been shown in cartilage [4] and cornea.[5]

When the metachromasia occurs in pathologic tissues, the deposition of cholesterol does not occur with normal serum cholesterol levels until the process has existed for some time (cholesterol in chronic inflammatory tissue [6]). When serum cholesterol is elevated, the deposition is more easily produced, and minor lesions will suffice to give rise to it (as seen in xanthoma in acute repair of tissue [6] and experimentally in tendons [7]). In this group it will be natural to include the Schüller-Christian granuloma in which the cholesterol content is high though the serum cholesterol is mostly normal.

Finally, the metachromasia may increase in organs in which it already occurs, and thereby accelerate the deposition of cholesterol. Such a phenomenon can be expected in the intima and the media of the aorta as a result of mechanical injury [8a] and in the granulation tissue of syphilitic mesaortitis.

Metachromatic tissue is always present in the human aorta and is described as due to the presence of chondroitin–sulfuric acid.[9] It is located in the intima and the luminal part of the media, places where cholesterol is found in the atheromatous vessels.[10] The metachromasia and the sulfuric acid that are present in the polyuronides are mainly found in the upper two thirds of the vessel.[11] This is the typical localization of the cholesterol deposits in the hypercholesteremic type of

3. Joel: Klin. Wchnschr. **3**:269, 1924.

4. Bürger, M., and Schlomka, G.: Ztschr. f. d. ges. exper. Med. **55**:287, 1925.

5. Martineau, J.: Les xanthomes vrais et les pseudoxanthomes infectieux, Thesis, Paris, Le Francois, 1927.

6. Faber, M.: Acta med. Scandinav. **118**:436, 1944.

7. Kusnetzowsky, N.: Virchows Arch. f. path. Anat. **263**:205, 1927.

8. Ssolowjew, A.: (*a*) Virchows Arch. f. path. Anat. **283**:213, 1932; (*b*) **261**:253, 1926.

9. Levene, P. A., and Lopez-Suarez, J.: J. Biol. Chem. **36**:105, 1918.

10. Ssolowjew, A.: Virchows Arch. f. path. Anat. **241**:1, 1923; **250**:259, 1924. Schultz, A.: Zentralbl. f. allg. Path. u. path. Anat. **239**:415, 1922.

11. Jorpes, E.; Holmgren, H., and Wilander, O.: Ztschr. f. mikr.-anat. Forsch. **42**:279, 1937.

atheromatosis as seen in xanthomatosis and untreated myxedema. Thus the anatomic findings do not exclude the hypothesis.

When cholesterol is fed to rabbits in sufficiently small amounts, the normal aorta will take up practically none of this cholesterol. After certain experimental procedures, however, an uptake can be shown to occur. The main feature of the type of injury that will deposit cholesterol in the aorta under these conditions is the subsequent reparative processes, and these should show an increase of metachromasia.

This increase has been demonstrated in epinephrine sclerosis by Erb.[12] Moreover, Ssolowjew[13b] showed that cauterizing the aorta from without produced a metachromatic zone. This zone was demonstrable for two to three months. In later experiments[13] he showed that the cauterization gave rise to increased deposition of cholesterol at the feeding of suboptimal doses of cholesterol only as long as the regenerative processes were active. No deposition could be produced coincident with the time when the metachromasia disappeared. Finally it may be mentioned that it is possible to produce aortitis experimentally with repeated injections of heterologous protein, probably accompanied by an increase of the metachromasia of the inflammatory tissue in the vessel. In this case suboptimal doses of cholesterol will result in deposition in the vessels, especially in the coronary vessels.[14]

In order to study this mechanism, an investigation was undertaken on the human aorta. As a measure of the carbohydrate–sulfuric acid ester content of the intima and media, the sulfate content of these tissues has been determined, on the assumption that all the sulfate is derived from these esters.

Fifty aortas have been examined. They came from autopsies at the Copenhagen County Hospital, Kommunehospitalet Copenhagen and the Medicolegal Institute of the University of Copenhagen. In all instances the aorta was prepared as soon as possible after the autopsy by removing the adventitia from the media and intima. These tissues were dried and hydrolyzed with fifth-normal hydrochloric acid for ten hours. The hydrolysates were extracted four times with ether; from the collected extracts cholesterol was determined by a quantitative Lieberman-Burchard reaction after alkaline hydrolysis.

The hydrolysates were filtered and evaporated to about 20 cc., and the sulfate was precipitated with barium chloride. When the sediment had settled, most of the supernatant was sucked off, and the sediment was transferred to a filter crucible, dried, ignited and weighed.

12. Erb, W.: Arch. f. exper. Path. u. Pharmakol. **53**:173, 1905.

13. Ssolowjew, A.: Ztschr. f. d. ges. exper. Med. **69**:94, 1930.

14. Schmitt: Virchows Arch. f. path. Anat. **296**:603, 1936. Brochs, H.: Experimentelle undersøgelser over lipoidaflejringen i coronararterierne hos kaniner, Thesis, Copenhagen, 1945.

The results are recorded in table 2, in which all the aortas are listed according to age in the four main groups: normal, hypertension,

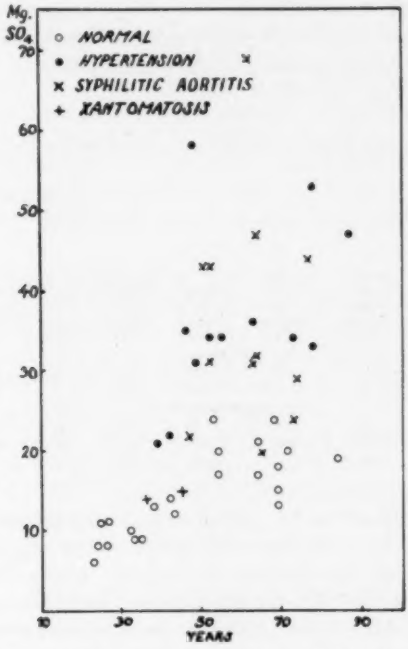
TABLE 2.—*Aortas Examined*

No.	Age	Sex	Weight of Dry Aorta, Gm.	Cholesterol in Aorta, Mg.	Sulfate in Dry Aorta		Blood Pressure	Diagnosis	Serum Cholesterol, Mg. per 100 Cc.
					Mg.	%			
Normal									
1	23	M	4	31	6	0.17	150/75	Rheumatic fever	...
2	24	M	3.5	20	8	0.24	.....	Bullet wound	...
3	25	M	3.9	24	11	0.26	.....	.....	...
4	26	M	5.2	37	11	0.21	.....	.....	...
5	26	F	3.6	34	8	0.23	.....	.....	...
6	32	M	3.2	77	10	0.30	.....	.....	...
7	33	F	3.2	25	9	0.28	.....	Poisoning	...
8	34	F	4.1	125	9	0.23	140/75	Chronic hepatitis	...
9	38	M	4.6	63	13	0.34	.....	Bullet wound	...
10	42	F	4.8	70	14	0.29	.....	.....	...
11	43	M	4.1	58	12	0.30	145/95	Poisoning	...
12	53	M	7.5	197	24	0.31	130/	.....	...
13	54	M	7.8	115	20	0.21	145/80	Chronic hepatitis	...
14	54	M	6.0	150	17	0.28	125/75	Duodenal ulcer	...
15	64	F	9.4	320	17	0.18	.....	Chronic hepatitis	...
16	64	F	13.2	275	21	0.16	140/80	.....	...
17	68	M	9.3	390	24	0.26	.....	Poisoning	...
18	69	F	10.8	490	15	0.14	140/80	Chronic hepatitis	...
19	69	M	11.1	355	13	0.12	120/70	.....	...
20	69	M	8.7	368	18	0.21	150/85	Cerebral hemorrhage	...
21	71	F	13.5	572	20	0.15	160/90	Chronic hepatitis	...
22	84	F	16.4	1,050	19	0.12	140/90	Heart disease	...
Hypertension									
23	39	M	6.9	125	21	0.30	.....	Coronary occlusion	...
24	42	M	6.9	145	22	0.32	.....	.....	...
25	46	F	8.6	250	35	0.40	200/110	Phlebitis	...
26	48	M	15.7	815	58	0.37	230/120	Heart disease	...
27	49	M	9.3	387	31	0.33	.....	Coronary occlusion	...
28	52	F	10.3	335	34	0.32	210/130	Pulmonary edema	...
29	55	M	10.9	283	34	0.31	250/140	Hypertension	...
30	61	F	16.8	1,150	36	0.22	190/110	.....	...
31	73	F	16.6	1,285	34	0.21	220/100	Chronic hepatitis	...
32	78	F	32.8	1,680	53	0.16	210/120	Hypertension	...
33	78	F	25.8	1,375	33	0.13	230/140	Cerebral hemorrhage	...
34	87	F	22.0	2,310	46	0.16	230/150	Arteriosclerosis	...
Syphilitic Aortitis									
35	47	M	8.3	325	22	0.27	.....	Syphilitic aortitis	...
36	51	M	28.0	356	43	0.15	.....	.....	...
37	52	M	16.2	690	43	0.27	210/120	.....	...
38	52	M	16.2	670	31	0.18	.....	.....	...
39	62	M	17.9	815	69	0.38	160/80	.....	...
40	63	M	19.0	706	31	0.16	165/100	.....	...
41	64	F	14.5	1,318	32	0.22	235/115	.....	...
42	64	M	19.0	860	47	0.25	205/85	.....	...
43	65	M	15.1	686	20	0.13	.....	.....	...
44	73	M	12.9	1,410	24	0.19	190/75	.....	...
45	74	F	24.6	1,278	29	0.12	210/90	.....	...
46	77	F	10.9	1,210	44	0.40	180/60	.....	...
Xanthomatosis									
47	36	M	4.4	253	14	0.31	.....	Xanthomatosis	400
48	45	M	7.5	430	15	0.20	.....	.....	398
Other Conditions									
49	17	M	3.7	19	15	0.42	.....	Poisoning	...
..	..	..	....	.....	..	....	.....	Congestive heart disease	...
50	17	F	2.7	33	9	0.33	.....	Peripheral vascular calcifications	...

syphilitic aortitis and xanthomatosis. Two aortas fall outside this grouping: One is that of a young girl with universal peripheral vascular calcifications; the other, that of a young man with congenital heart

disease. The latter had a relatively high sulfate content, while the first was normal.

The chart shows the relation between the total sulfate and age. It will be seen that in normal states the sulfate content of the aorta increases with advancing age, reaching a maximum at the age of 60, after which it remains constant. In hypertension there is shown a similar rise with age, but nearly all values lie above those for the normal group



The sulfate content of the aorta in relation to age.

in the same age group, and frequently above the highest normal values. The same is found in regard to syphilitic aortitis, though here the values are more scattered, with some relatively low values. Thus an increased amount of sulfate is found in these two forms of aortic lesions in which the deposition of cholesterol must be regarded as being accelerated on account of an increase in the tissue factor. Conditions are different in the 2 xanthomatous aortas. Each showed an entirely normal sulfate content. This was to be expected if it was the increase in the available cholesterol that determined the rate of deposition.

A somewhat different picture is seen when one is considering percentages. In the normal group there is a slight increase up to the age of 40, followed by constant values until the age of 60, when a distinct fall occurs. In the hypertensive group, compared with the normal group, the percentage is higher and there is the same tendency toward lower values in old age, though hardly as pronounced as in the normal group. No regularity is to be found in the aortas of persons who had syphilitic aortitis, nor is that to be expected, since the activity of the syphilitic processes must determine the sulfate content. Some of the aortas studied must undoubtedly be regarded as healed, and the predominant chemical residual lesion is a strong calcification. This applies especially to nos. 40 and 43.

TABLE 3.—*The Sulfate Content of the Aorta Corrected for Calcium and Cholesterol*

No.	Weight of Dry Aorta, Gm.	Cholesterol in Aorta, Mg.	Calcium in Aorta, Mg.	Corrected Weight of Aorta, Gm.	Sulfate in Dry Aorta: Mg.	Sulfate in Dry Aorta: Per Cent of Total Aorta	Sulfate in Dry Aorta: Per Cent of Aorta Corrected for Cholesterol and Calcium
			Normal				
8	4.1	125	31	3.8	9	0.23	0.24
20	8.7	268	106	8.0	18	0.21	0.24
			Hypertension				
27	9.3	287	99	8.7	31	0.33	0.36
33	25.8	1,375	3,410	15.5	22	0.13	0.25
34	22.0	2,310	2,112	14.2	46	0.16	0.32

The rise observed in the sulfate content with advancing age will thus be due mainly to the increasing weight of the organ. There are several reasons for this increase of weight. Among these are the calcium and cholesterol deposits which do not participate in the growth and metabolism of the vessel. With the assumption that the cholesterol measured represents the total lipid and that the calcium was deposited as three molecules of calcium phosphate to one molecule of calcium carbonate ($3\ Ca_3\ (PO_4)_2,\ CaCO_3$), the "metabolic active" tissue can be calculated. This correction is at any rate not too high in view of the fact that actually one finds considerable amounts of lipids besides the cholesterol. Applying the correction as seen in table 3, one sees that the percentage content of sulfate is at least at the same level in the senile vessels as in the younger vessels. The active part of the vessel thus appears to contain a rising percentage of sulfate during the whole age interval investigated.

COMMENT

In the foregoing pages an attempt has been made to establish a common rule for a series of extracellular cholesterol depositions and to confirm this rule by the study of a single organ. The human aorta gives in the main confirmation of the theory advanced. As mentioned, sulfuric acid esters are found in places corresponding to the cholesterol deposits, and the increasing amount of cholesterol with rising age corresponds to a rising amount of sulfate. In cases of hypertension one finds by absolute measurement and by percentage an increase in sulfate corresponding to the increased rate of deposition of cholesterol. The same is more or less true of the majority of cases of syphilitic aortitis, but here the activity of the inflammation seems to determine the sulfate content. One might perhaps have expected a somewhat higher sulfate content in old age, considering the distinctly increasing rate of cholesterol deposition with age.

There remains a discussion of why the cholesterol is deposited more easily in the sulfuric acid ester–containing tissue than in other tissues.

It is generally accepted that the cholesterol is not synthesized at the place of deposition but is brought into the tissue from the plasma. The cholesterol which enters the tissues will thus be in the form of the serum lipoproteins. The question is: How will the lipoproteins react with the sulfuric acid esters to give rise to cholesterol precipitation? The investigations of Chargaff [15] may suggest an answer. When heparin is allowed to act on lipoproteins, the heparin occasionally enters the lipoprotein. In the majority of cases, however, there occurs a liberation of the lipid, the heparin assuming the place of the lipid in the protein. The latter mechanism seems to fit the conditions found in the wall of the aorta and in other places where cholesterol is deposited extracellularly. It must, however, be mentioned that the serum lipoproteins will not release their lipids on being treated with heparin in their native state.

One phenomenon deserves further mention. The absolute sulfuric acid content of the aorta increases with age, but the percentage content hardly shows the same rise. Accordingly, one would expect that the rate at which the cholesterol is deposited would rise at a rate that would be not much higher than the rate of the increase in weight. This is, however, not the case. The weight of the dried intima and media of the aorta will rise following the equation log. weight $= 0.3171 + 0.0100 \times$ age,[16] while the cholesterol content follows the equation log. cholesterol $= 0.9792 + 0.0241 \times$ age. This means

15. (*a*) Chargaff, E.; Ziff, M., and Cohen, S. S.: J. Biol. Chem. **136**:257, 1940. (*b*) Chargaff, E., ibid. **142**:491, 1942.

16. Faber, M., and Lund, F.: To be published.

that the weight of the vessel increases only about five times during life, while the rate of the cholesterol deposition during the same time rises ten to twenty fold.

It is possible that this increased rate of deposition is due to changes in the lipoproteins of the serum.[17] Reference is here made to a frequently mentioned but still rather vague phenomenon, the cholesterolytic property of serum. Several authors have indicated that while cholesterol when added to serum from younger persons increases the amount of cholesterol dissolved probably as lipoprotein, the same is rarely true in regard to older persons, in whose case one may even observe a precipitation of the cholesterol already dissolved in the serum. This negative cholesterolytic property should be most pronounced in cases of hypertension. The phenomenon, however, requires further study. Since ample amounts of free cholesterol may be found in vessels with a high cholesterol content—even in crystalline form—it may be that a negative cholesterolytic property of the serum accelerates the deposition of cholesterol.

SUMMARY

On the basis of the literature it is shown that the organism contains a number of extracelluiar cholesterol deposits and that these are always to be found in tissues which beforehand contain substances that can be stained metachromatically. It is therefore possible that these substances are responsible for the depositions of cholesterol.

In the human aorta one finds an increasing content of sulfate as a measure of the metachromatically stainable carbohydrate–sulfuric acid esters with advancing age. Higher values are found in cases of hypertension and in most cases of syphilitic aortitis, corresponding to the increased cholesterol contents. The sulfate content of the xanthomatous aorta is normal.

The paper discusses the details of the mechanism involved in the deposition of cholesterol in a tissue containing sulfuric acid esters of the kind mentioned.

17. Alvaretz and Neuschloss: Klin. Wchnschr. **10**:244, 1931. Eck, and Desbordes: Compt. rend Soc. de biol. **118**:498, 1935. Obrecht: Ueber das cholestrolytische Vermögen des Blutserums im alter und bei Hypertension, Thesis, Bern, 1941.

THE HUMAN AORTA

Influence of Obesity on the Development of Arteriosclerosis in the Human Aorta

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OBESITY is quite often the first visible link in the chain of events which after some years will give rise to signs of arteriosclerotic disease.

It is a question how great an influence the obesity in itself will have on this development—whether the sclerosis is a direct result of the obesity and the biochemical and other changes that follow this condition, or whether the obesity affects the vessels through a more complicated mechanism. A few workers have tried to study this problem.

In a study of 1,250 aortas Wilens [1] correlated the weight of the patient with the degree of arteriosclerosis. The sclerosis was evaluated by macroscopic appraisal at the autopsy, taking into account not only the findings in the aorta but in most cases the state of the other vessels also. He reported that as compared with the degree of sclerosis found in patients of normal weight the sclerosis observed in the obese was greater and that in the emaciated less. In a later work [2] he extended these studies and showed that premortal emaciation is followed by a decrease of the sclerosis of the aorta. Similar results were obtained by Eskola [3] using a similar technic. The value of these studies is somewhat diminished, however, by the great difficulty encountered when a quantitative evaluation of the findings has to be made on the basis of the macroscopic appearance of the vessel.

A more exact evaluation of the sclerosis should be possible if a quantitative study is made of any of the substances of the wall of a vessel, especially if these substances can be shown to bear a well defined relation to the sclerotic changes. On the basis of determinations

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1. Wilens, S. L.: Arch. Int. Med. **79**:129, 1947.
2. Wilens, S. L.: Am. J. Path. **23**:793, 1947.
3. Eskola: Duodecim **64**:443, 1948.

of cholesterol and calcium Rosenthal [4] was not able to find any interdependence between obesity and the arteriosclerotic changes. Bjørnsson,[5] who used a somewhat similar technic, noted a slight but not significant increase of the sclerotic changes in obesity.

It is generally accepted that obesity in many cases will be complicated by diseases which in themselves may evoke an increased or rather an acceleration of sclerosis. The most important of these diseases is hypertension. The evidence that increased blood pressure will produce sclerotic changes of both the central and the peripheral arteries and of the veins has recently been summarized by Moschowitz.[6] The question still remains whether obesity is able to increase the sclerosis when hypertension is present. In Wilens' series the effect of weight was evident also in the hypertensive group.

Diabetes mellitus is another complication of obesity which may influence the rate of sclerosis. The mechanism of the diabetic sclerosis is not known, probably the causes are complex. In the sclerosis seen late in diabetes, hypercholesteremia must, however, be taken into account, even if it has been of short duration.

Hypercholesteremia will probably also be the determining factor for the sclerosis seen in Cushing's disease and in myxedema. The same will be the case in hereditary hypercholesteremia and in xanthomatosis, in which a relative increase in the cholesterol content of the aorta is a typical finding (Faber [7]). In a series of 80 soldiers dying from coronary occlusion French and Dock [8] observed some obesity in 73. The description given of the aortas is however so close to the type found in hypercholesteremia that these aortas cannot be accepted as showing a direct effect of obesity on vascular sclerosis so long as no serum cholesterol determination is reported.

The moderate degree of hypercholesteremia reported by Gildea, Kahn and Man [9] and later by Kornerup [10] in persons of pyknic type must, however, be considered. These persons will presumably for the most part belong to the obese group, and this will influence the degree of sclerosis seen in cases of obesity.

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4. Rosenthal, S. R.: Arch. Path. **18**:473 and 660, 1934.

5. Bjørnsson, J.: Arteriosclerosis: A Chemical and Statistical Study, Copenhagen, 1941.

6. Moschowitz, E.: Vascular Sclerosis, New York, Oxford University Press, 1942.

7. Faber, M.: Acta med. Scandinav. **125**:210, 1946.

8. French, A. J., and Dock, N.: J. A. M. A. **124**:1233, 1944.

9. Gildea, E. F.; Kahn, E., and Man, E. B.: Am. J. Psychiat. **92**:1247, 1936.

10. Kornerup, V.: Familiaer hypercholesterolaemi og xanthomatose, Thesis, Copenhagen, 1948.

In comparing the cholesterol content of the aorta with the serum cholesterol it has not been possible to show any correlation so long as the serum cholesterol remained inside the normal range. With serum cholesterol above the normal levels, however, such a relation could be found.[7] Studies on the serum cholesterol of patients with clinical signs of coronary vascular disease, angina pectoris and coronary occlusion indicated, however, that the lower the serum cholesterol the higher the age at which the coronary sclerosis became manifest, a finding now substantiated by Morrison and co-workers.[11] These results indicate that changes of serum cholesterol level inside the normal ranges perhaps during a longer period can be of importance for the rate at which cholesterol is deposited in the wall of the vessel.

Of great interest is the question whether a diet low in cholesterol will decrease the concentration of cholesterol in the serum. Studies on the rice diet show that this can be the case.[12] However, other diets not low in cholesterol but low in calories seem to give the same result. The low incidence of sclerosis found in the Chinese by Oppenheimer[13] and later by Snapper[14] could be explained by these facts. Weiss and Minot[15] claimed that patients dying of pulmonary tuberculosis show less sclerosis than was to be expected. This would follow from the poor nutritional state, perhaps complicated by the persistent hypotension seen in chronic infectious diseases.

We have found the problem of the relationship of obesity and arteriosclerosis still undecided and have therefore, in a series of cases, tried to get as exact figures for the relationship as possible.

To grade a sclerotic vessel macroscopically post mortem, even when the age of the subject is known, is extremely difficult, and there can be no doubt that each arterial system must be considered by itself. A parallel change in the different parts of the arterial system cannot be expected. This is shown in the preponderance of males among patients with coronary occlusion in the younger age groups and in the earlier and more marked calcification seen by roentgenogram in the abdominal aorta in women.[16]

We have restricted this study to the intima and media of the total aorta, and as a basis for the evaluation we have studied three factors —the dry weight, the cholesterol content and the calcium content.

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11. Morrison, L. M.; Hall, L., and Chaney, A. L.: Am. J. M. Sc. **216**:32, 1948.
12. Kempner, W.: Bull. New York Acad. Med. **22**:358, 1946.
13. Oppenheimer, F.: Chinese M. J. **39**:1067, 1925.
14. Snapper, I.: Chinese Lessons to Western Medicine, New York, Interscience Publishers, Inc., 1941.
15. Weiss, S., and Minot, S. R.: Nutrition in Relation to Arteriosclerosis, in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, pp. 237-248.
16. Petersen, G. F.: Acta radiol. (supp.) **39**:1, 1941.

MATERIAL

The aortas studied were collected at autopsies in the hospitals of Copenhagen and in the Medicolegal Institute of the University of Copenhagen. All the studied cases in which sufficient data were available have been used except the following groups: all cases of syphilitic aortitis and cases in which valvular heart diseases was present; cases in which hypercholesteremia or diabetes mellitus was present, and cases of Cushing's syndrome or myxedema. Cases described in previous publications have been used when sufficient data were available. The majority of the cases of normal status were collected at the Medicolegal Institute and consisted primarily of cases in which sudden death occurred as a result of an accident. The rest of the material has been collected so as to get the greatest number of cases with hypertension and obesity. Most of the hospital patients died less than forty-eight hours after admission. Of the rest, only a few had wasting diseases. We therefore think this material will not be affected by Wilens'[2] finding that premortal emaciation should decrease the sclerosis. The diet, especially the lipid content of the Danish diet, has not been changed during the four years these cases have been accumulated to a degree that will make them incomparable.

PROCEDURE

The total aorta from 1 cm. above the aortic valve to the bifurcation was prepared free of adventitia soon after the autopsy. The error introduced by a slightly inaccurate dissection will turn up only in the weight, the greatest part of the cholesterol and the calcium being in the luminal part of the media and in the intima. The tissue was cut up with scissors and dried in a vacuum over sulfuric acid for twenty-four hours, and the weight determined. This weight has been used throughout, although it is not the correct dry weight of the tissue, but somewhat higher.

The dry tissue or an aliquot was hydrolyzed for three hours in fifth-normal sodium hydroxide and extracted four times with ether, and the cholesterol was determined from the collected ether extracts by a quantitative Lieberman-Burchard reaction.

The calcium was determined in an acid hydrolysate of the tissue by precipitation with oxalate at p_H 5.8 and permanganate titration.

In most of the cases the weight and the height were obtained in the hospital or at autopsy. Only in a few cases did we have to rely on the simple evaluation, normal, obese and very obese. The normal weight of each patient was calculated according to the tables given by Fisk and Crawford,[17] this calculation taking into account the height and the sex of the patient. No correction was made for the fact that the figures of Fisk and Crawford were obtained on fully dressed persons, because the lack of shoes probably would compensate for the lowering of the weight due to lack of clothes. Obesity has in this study been defined as a weight more than 10 per cent above the calculated normal weight. A group of very obese persons with weights more than 25 per cent above the normal will be considered separately.

The highest blood pressure measured during the terminal hospitalization has been used. In some cases earlier measurements were available and have been of use in cases in which the premortal blood pressure represented the blood pressure

17. Fisk, E. L., and Crawford, J. R.: How to Make the Periodic Health Examination, New York, The Macmillan Company, 1927.

during shock. Blood pressures above 160 mm. of mercury systolic and 90 mm. diastolic have been registered as hypertension.

In a relatively large group no data on blood pressure were available, especially in the cases of sudden death. In these, however, the heart weight was used. We have classified such cases as cases of hypertension when the heart weight was more than 2.5 times the standard deviation, that is, more than 100 Gm. higher than the normal weight when sex and height were taken into consideration.[18] In the cases in which the heart weight and the blood pressure were known, an elevated blood pressure was considered more significant than the heart weight. This discrepancy was seen in only few cases however.

TABLE 1.—*Distribution of Material (408 Aortas) According to Sex, Blood Pressure and Body Weight*

	Normal Blood Pressure		Hypertension		
Sex	Normal Weight	Obesity	Normal Weight	Obesity	Total
Male	141	22	39	38	240
Female	77	19	38	34	168
Total	218	41	77	72	408

TABLE 2.—*Distribution of Material (408 Aortas) According to Age, Blood Pressure and Body Weight*

	Normal Blood Pressure		Hypertension		
Age	Normal Weight	Obesity	Normal Weight	Obesity	Total
Below 20	10	0	0	0	10
20-29	32	0	1	0	33
30-39	47	10	7	1	65
40-49	28	11	12	12	63
50-59	38	9	10	16	73
60-69	39	9	18	21	87
70-79	17	1	20	18	56
80-89	6	1	6	4	17
Above 90	1	0	3	0	4
Total	218	41	77	72	408

A total of 408 aortas were used in this study. The distribution in body weight and blood pressure groups according to sex is shown in table 1. The distribution according to age is shown in table 2.

The great increase in the degree of sclerosis with rising age makes it necessary in some way to eliminate the age factor from the evaluation of the sclerosis. It has been shown by Bjørnsson [5] that the logarithm of the cholesterol and calcium content of the vessel wall rises rectilinearly with age, and the same is found for the dry weight of the

18. Zeek, P. M.: Arch. Path. **34**:820, 1942.

tissue. To eliminate the effect of age, we have calculated the formula for this line for the three factors studied, and the evaluation of the sclerosis will be on the basis of the deviations from this line as found in each aorta.

These deviations have been used for the construction of distribution curves, and the distribution curves of the different groups of cases will be compared.

WEIGHT

The dry weight of the normal intima and media of the total aorta is found to vary between 2 and 26.5 Gm. On the basis of the normal material the formula for the rise in weight with age when the weight is measured in grams is found to be: log. weight $= 0.3171 + 0.100 \times$ age.

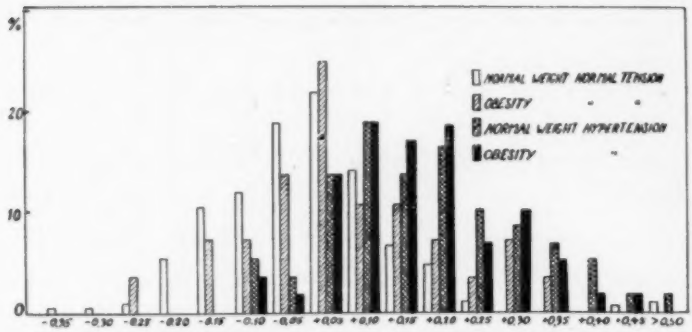

Chart 1.—The distribution curves for the dry weight of the intima and media of the aorta around the calculated normal line in the four groups studied.

In chart 1 is seen the distribution curve for the weight in the four groups under study when the group with normal weight and normal blood pressure is used for the calculation of the standard line. The spread around the mean is fairly large, but the cases with obesity and normal blood pressure show a distribution curve of exactly the same mean and spread as is found for the group with normal weight. In the two groups with hypertension the results are similar. Both show a displacement toward higher values, but the displacement is the same in both groups with the result that the two curves are practically identical.

These results show that hypertension will produce a rise in the weight of the wall of the aorta to values higher than was to be expected at the age of death. It is, however, not possible to show any effect specific for the obesity when the material is broken up according to blood pressure.

CHOLESTEROL

The cholesterol content of the aorta was found to vary between wider limits than the weight of the tissue. The lowest cholesterol content found was 11 mg. and the highest 3,130 mg. By means of the normal material the formula for the cholesterol content of the vessel measured in milligrams is found to be: log. cholesterol = $0.9792 + 0.0241 \times$ age. The distribution curves for the four groups are found in chart 2. As was the case with the dry weight, the distribution curves for the cholesterol in the groups of normal weight and obesity show coincidence both in maximum and in spread in the group with normal blood pressure. In this regard also the cases of hypertension show a displacement toward higher values as was to be expected with the normal weight and the obesity groups behaving identically.

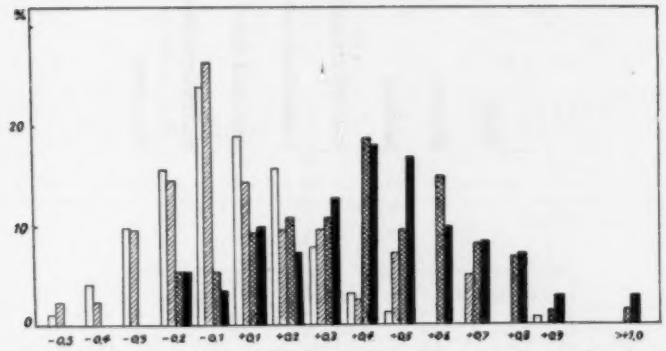

Chart 2.—The distribution curves for the total cholesterol content of the intima and media of the aorta around the calculated normal line for the four groups studied. See chart 1 for key.

The result of the study of the cholesterol content of the aorta thus followed that of the dry weight in not showing any effect of obesity as such.

CALCIUM

As was to be expected, the spreading of the calcium values was still greater than was that of the cholesterol values. The lowest value found was 4 mg. and the highest 3,410 mg. The formula for the rise in calcium content in relation to age will therefore show a steeper rise than was found in the other curves. Expressed as milligrams, the formula is found to be: log. Ca. = $0.3060 + 0.0318 \times$ age. The deviations from this line are found in chart 3. The spreading of the values around the calculated line is somewhat greater than with the dry

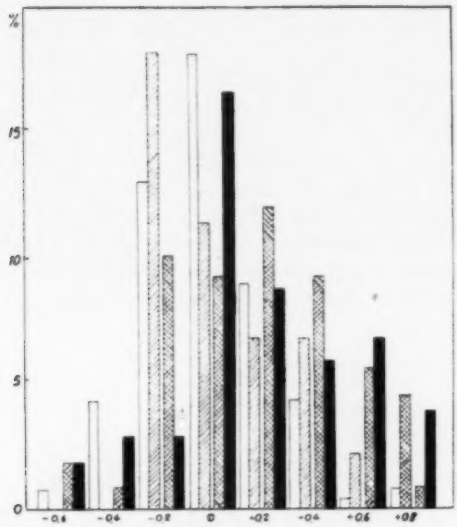

Chart 3.—The distribution curves for the calcium content of the intima and media of the aorta around the calculated normal line for the four groups studied. See chart 1 for key.

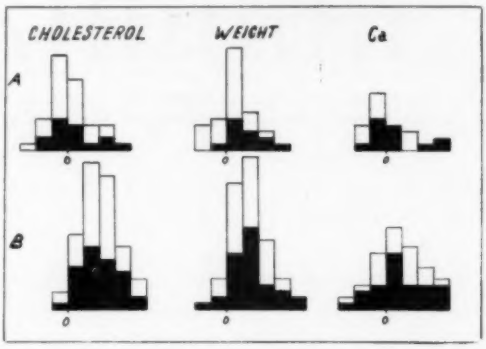

Chart 4.—The distribution of the very obese (*o*) among all the obese in the distribution curves from charts 1 to 3 in two series: *A*, normal blood pressure; *B*, hypertension. Black represents weights more than 25 per cent above the normal; white, weights between 10 and 25 per cent above normal.

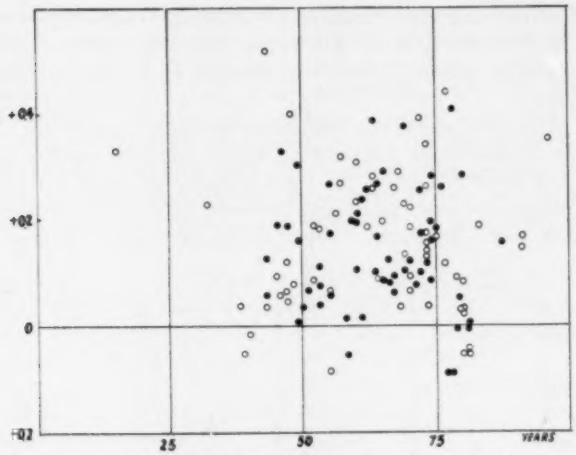

Chart 5.—The deviations in log. dry weight from the calculated normal line in relation to age. White circles represent deviations associated with normal body weight; black circles, deviations associated with obesity.

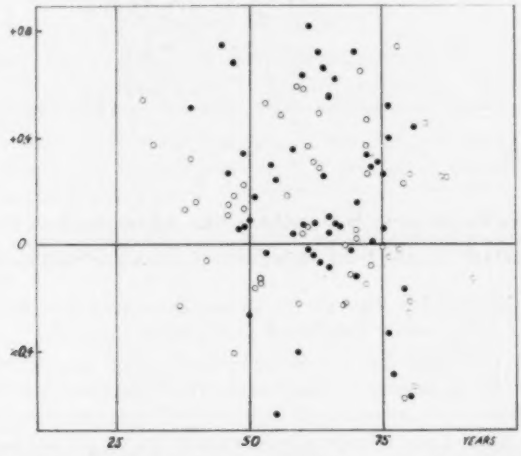

Chart 6.—The deviations in log. cholesterol from the calculated normal line for the hypertensives in relation to age. White circles represent deviations associated with normal body weights; black circles, deviations associated with obesity.

weight and the cholesterol. Remarkable is the relatively large number of normal and low normal values found in cases of hypertension. The relative positions of the distribution curves are, however, as found with the other factor studied, with no sign of any specific effect of obesity.

It might be claimed that the limits for obesity have been drawn too low in this study and that an effect could be masked in this way. To settle this point, the obese material has been divided into two groups, one with overweight between 10 and 25 per cent and the other with overweight more than 25 per cent. As seen from

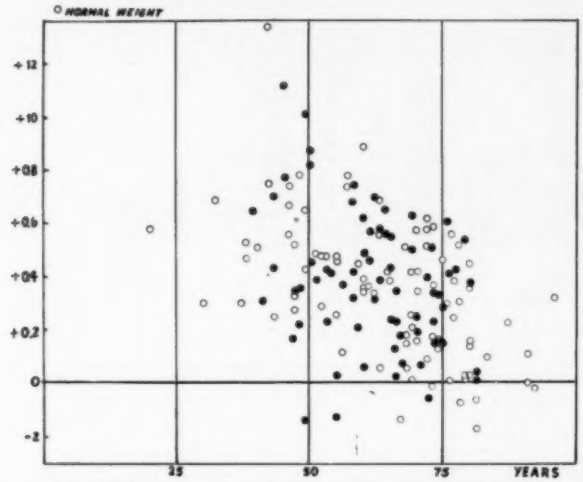

Chart 7.—The deviations in log. calcium from the calculated normal line for the hypertensives in relation to age. White circles represent deviations associated with normal body weights; black circles, deviations associated with obesity.

chart 4, this division does not invalidate the conclusions drawn, the very obese being evenly distributed in the obese material.

In this material it has not been possible to show any variations on account of sex or height. An analysis of the blood pressures measured did not show any increase in the degree of sclerosis depending on how much the blood pressure was elevated above the upper normal limits.

In the distribution curves for the cases of hypertension it was shown that some of the values found were fairly low, often below the normal mean. These low values may have a different significance than the higher values found in most of the cases of hypertension. One possibility is that this was an effect of age.

In chart 5 is seen the deviation from the calculated line in relation to age for the dry weights of the aortas of the hypertensive group. No relation to age can be seen. In regard to cholesterol, however, there can be shown a definite age dependence, as seen in chart 6. When the material is divided into age groups an interesting difference is found. Up to the age of 50 years even the cases with the least deviation are found far away from the calculated line. In the age group between 50 and 75 years, the largest deviations are missing, and the smallest approach the normal line. In the group above 75 years this movement toward the normal is continued, and when 80 years is passed a normal distribution is found.

The behavior of the calcium is seen in chart 7. The distribution of the values in the figure resembles mostly the distribution seen in regard to the weight. However, the number of normal, and especially of low normal, values seems to rise with increasing age.

This normalization of the values with increasing age can be explained only if it is assumed that the hypertension which is seen in these very old people is of another type than the one met with in the younger age groups. The elevation of blood pressure is moderate, in most cases below 200 mm. systolic, and is presumably to be considered a result of the normal sclerosis of the vessels and not as a disease in itself.

SUMMARY

The influence of obesity on arteriosclerotic changes has been studied on the basis of determinations of aortic dry weight, cholesterol and calcium in 400 aortas.

The rise of these three factors with age has been calculated. It can be shown that hypertension gives a rise above what should be expected according to age.

Obesity itself, however, has no effect on any of the factors studied when the presence of hypertension is taken into account.

A PREINVASIVE CARCINOMA OF THE UTERINE TUBE

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AND
G. H. GARDNER, M.D.
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PRIMARY carcinoma of the uterine tube is a rare lesion. In all of the reported cases the lesion was relatively well advanced when discovered, except for a case reported by Mitchell and Mohler [1] in 1945. In their case the tumor was discovered during routine examination of small segments of tube excised for the purpose of sterilization. Subsequent total hysterectomy and bilateral salpingo-oophorectomy were performed. However, the Mitchell-Mohler specimen is a well developed small tumor, since it had almost obliterated the lumen of the tube, had extended into the muscle and had invaded lymph spaces and small blood vessels.

The carcinoma of the tube to be presented here was discovered accidentally. The essential pathologic process was pelvic endometriosis, and total hysterectomy and unilateral salpingo-oophorectomy were performed. The tube and ovary were not included in the tissues sent to the hospital laboratory but were diverted to our special study of broad ligaments. During microscopic examination, attention was attracted to a particular area in the tube because of several mitotic figures in one microscopic field (mitotic figures are extremely rare in tubal epithelium). Further examination made it obvious that there was a minute preinvasive carcinoma of the uterine tube. The tumorous area occupied approximately a tenth of the total cross section area of the endosalpinx. There was histologic evidence of follicular salpingitis, with its characteristic fenestration of sealed plicae in the endosalpinx. The tumor had replaced the epithelium of three of these glandlike spaces and portions of several others. There was no invasion of the underlying musculature, and no invasion of blood vessels or lymph spaces. In fact, there was no evidence of invasion at all.

The epithelium was piled up, and in some areas truly stratified. Apparently the tumorous epithelium was progressing along the lumen of several of the glandlike spaces, and in each there was a point of abrupt transition from tumorous to normal epithelium. Mitotic figures

From the Departments of Obstetrics and Gynecology, Northwestern University Medical School and Wesley Memorial Hospital.

1. Mitchell, R. M., and Mohler, R. W.: Am. J. Obst. & Gynec. **50**:283, 1945.

were frequent. Many of these mitotic figures were abnormal in appearance, tripolar figures being common. The nuclei in general were hyperchromatic, and the nucleoli were prominent. The nuclei varied in size and shape, and a few truly giant nuclei were present; also an occasional giant mitotic figure was observed.

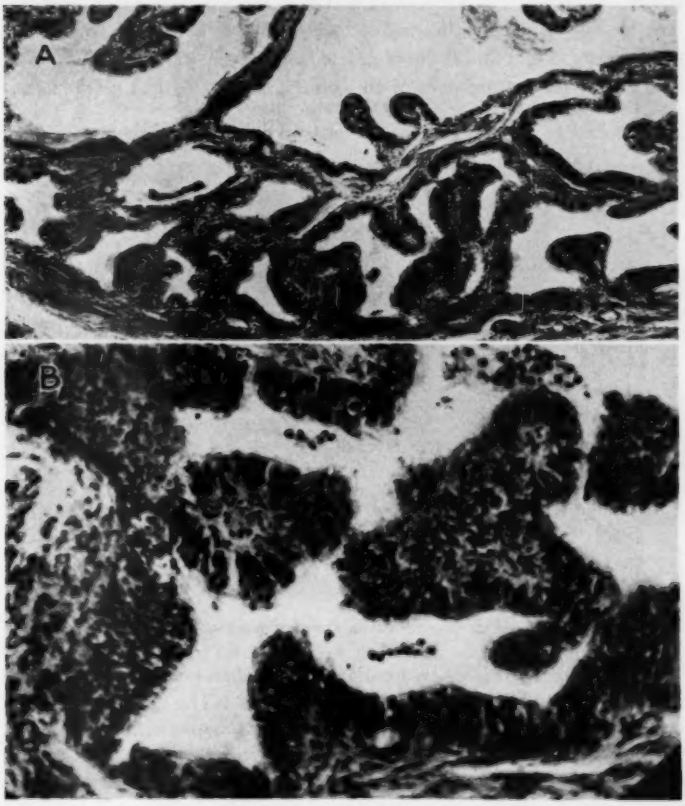

Fig. 1.—*A*, low magnification of preinvasive carcinoma of the uterine tube, showing total extent of carcinomatous area (thicker and darker-staining epithelium). *B*, medium magnification, showing stratification and nuclear variations.

Both the secretory and the ciliated types of cells had apparently participated in this carcinomatous process. There was, however, some variability in the appearance of different areas. In some, the cytoplasm of the ciliated cells still stained lightly, and the nuclei were somewhat

vesicular and had the usual shape; however, they were approximately twice normal size. The secretory cells were markedly enlarged, crowded and hyperchromatic. Mitotic figures were noted in these same areas, but the type of cell which was undergoing mitosis could not be determined.

In other areas the cytoplasm of all cells stained more deeply. The nuclei of ciliated cells were elongated and frequently irregular in shape. The amount of chromatin seemed to be increased. In some areas cells of secretory origin and those of ciliated cell origin were not distinguishable except for the presence of cilia on the free or luminal border of the latter.

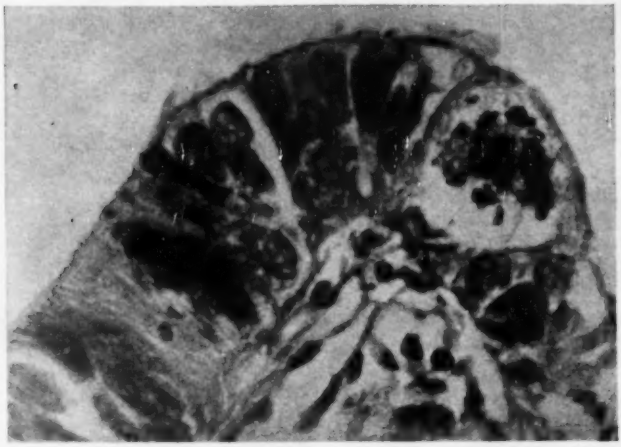

Fig. 2.—High magnification, showing giant mitotic figure.

In still other areas dedifferentiation had taken place and there were no cilia. In some areas anaplasia was marked. The nuclei were small and relatively uniform in size, but the gross increase in the size of the nucleolus or nucleoli was particularly obvious. Elsewhere a small portion of the tissue showed definite nuclear pleomorphism; here the nuclei were hyperchromatic and varied markedly in size and in shape.

The noncancerous portion of the tubal epithelium was quite high, with the usually varying proportion of ciliated and secretory cells. In some areas the epithelium was pseudostratified. In a few areas there was a piling up of the cells to produce intraluminal "tufts" or small "papillae." There were a few large, atypical-appearing cells with bizarre-shaped, atypical nuclei. One mitotic figure was noted. These findings denote a moderate degree of endosalpingeal hyperplasia.

Subsequent to the discovery of this small tumorous area, multiple blocks were made from the remaining portion of the tube, and more sections were made from the original block. The tumor was found to extend for a very short distance. No evidence of tumor was found in any of the other blocks.

The discovery of this tumor was, of course, purely fortuitous. Since primary carcinoma of the fallopian tube is extremely rare, it is not expected that it will be found in such an early preinvasive stage in many cases.

Our patient is under observation; it is now eight months since the operation; she is well and free from objective evidence of recurrence; no further treatment is planned.

Laboratory Methods and Technical Notes

A PRACTICAL DEVICE FOR DEMONSTRATING AIR EMBOLISM

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CLEVELAND

THE QUANTITATIVE and qualitative demonstration of air or other gases that may be present in the cardiac ventricles, the pleural sacs or other cavities of the body must not be neglected in the course of forensic autopsies. Furthermore, it is a questionable practice to arrive at the diagnosis of air embolism, of pneumothorax and like conditions by retrospection, or, so to say, circumstantial evidence. The method which is recommended in the textbooks of pathology or the handbooks for autopsies,[1] i. e., the opening of the cavity under water, is rather primitive and not always practicable even when the proper precautions are taken.

In the many autopsies at the Coroner's Office in Cleveland (more than 1,500 autopsies in the last four years) the need for a simple apparatus for making such demonstrations compelled me to develop the one herein described. This device is constructed of such materials as are readily available in any laboratory and is so simple that its use should present little difficulty.

DESCRIPTION OF THE DEVICE

As shown in figure 1, the apparatus consists of the following parts:

A. One wide mouth glass bottle (2 or 3 ounce [60 to 90 cc.] capacity) fitted tightly with a two hole rubber stopper.

B. Two sections of glass tubing of approximately 3 mm. inside diameter, each bent at an angle of 120 degrees. One of these sections should be longer than the other. The shorter one should reach just through the stopper and be even with the inner surface of the stopper. The longer one should reach to within 1 or 1.5 cm. of the bottom of the flask. Both tubes should fit tightly into the holes of the stopper.

C. One separatory funnel (60 to 100 cc. capacity, pear shaped) connected to the longer section of bent glass tubing by rubber tubing 100 cm. in length (*F*). In my experience an amber, pure gum rubber tubing such as is used on blood diluting pipets has proved satisfactory.

D. One transfusion needle, no. 14 or 15 gage, 4 or 5 cm. in length, connected to the shorter glass tube by a short section of rubber tubing not exceeding 5 cm. in length (*F*).

From the Cuyahoga County Coroner's office.

1. Anderson, W. A. D.: Pathology, St. Louis, C. V. Mosby Co., 1948, pp. 126 and 127. Boyd, W.: A Textbook of Pathology, ed. 5, Philadelphia, Lea & Febiger, 1947, pp. 83 and 476. Saphir, O.: Autopsy Diagnosis and Technique, ed. 2, New York, Paul B. Hoeber, Inc., 1946, pp. 75 and 189. Moritz, A. R.: Pathology of Trauma, Philadelphia, Lea & Febiger, 1942, p. 132. Gradwohl, R. B.: Clinical Laboratory Methods and Diagnosis, ed. 4, St. Louis, C. V. Mosby Co., 1948, vol. 2, pp. 1855 and 1866.

E. Two pinchcock clamps, one for each length of tubing. They may be of the spring type or of the household syringe type. The latter will prove advantageous if the gas collected is to be transported for analysis.

The entire system is filled with liquid petrolatum so that when the funnel is at a level with the upright bottle the oil fills only about one half of the funnel.

TECHNIC

In operation the funnel is first raised to a position 30 or 40 cm. above the level of the upright bottle (position one in fig. 2). All the cocks are opened and the position is retained until every trace of gas has been driven from the system through the needle which is thereby coated on the inside by a film of oil. After

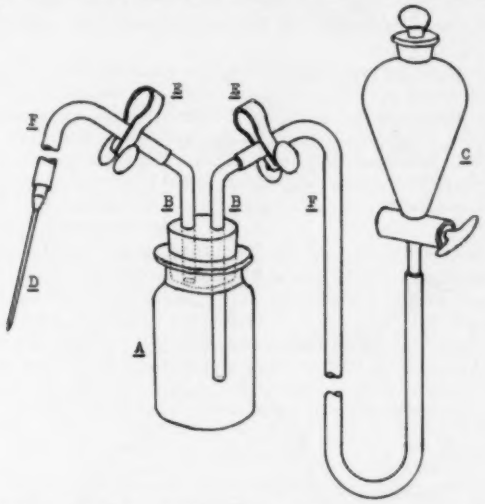

Fig. 1.—Apparatus for the demonstration of air embolism. See text for the explanation of this diagram.

all air has been expelled, the cocks are closed and the funnel lowered once again to its original position.

As a precautionary measure and control, the airtightness of the whole system should be tested before operation. This is done by inserting the needle into musculature or skin and attempting aspiration in the manner described in the next paragraph.

To make the test, the bottle is inverted and the needle inserted in the cavity in question. When the needle is in position, all cocks are opened. The funnel is lowered about 70 to 90 cm., or until adequate suction is created. Thereby the contents of the cavity are aspirated. These may consist of air or other gases, either pure or mixed with blood or other liquid. Any gas or liquid entering this system may be observed through the wall of the short bent glass tubing. In case of a positive test, gas bubbles will collect in the bottle above the level of the oil. If desired, this gas may now be saved for further examination by closing all the the cocks and returning the bottle to its upright position.

As a less satisfactory substitute for this apparatus, I have used a 30 cc. glass syringe which was half-filled with water and fitted tightly to a no. 15 gage needle. All air bubbles must have been removed and the whole thing checked for air-tightness. The test may then be made by inserting the needle as described in the foregoing paragraph and applying slow and careful suction. Here the principal difficulty lies in the inability to control the suction applied, so that under excessive negative pressure the gases normally dissolved in the blood may be liberated.

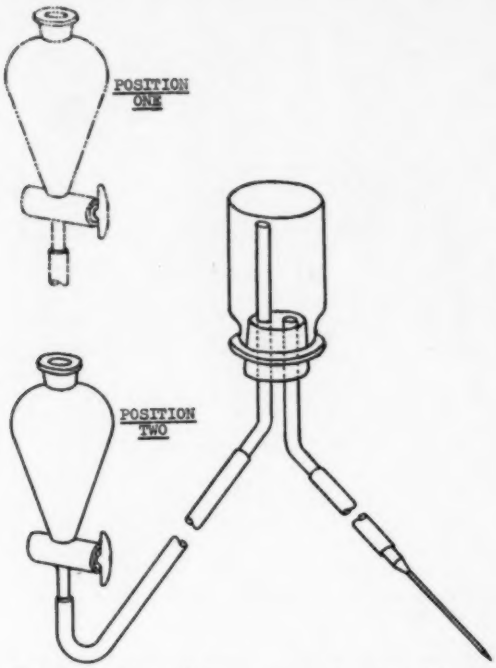

Fig. 2.—The two positions of the separatory funnel used in the test for air embolism.

COMMENT

To illustrate the value of an adequate method I cite here 5 cases of air embolism which came to my attention recently.

Case 1.—A 39 year old Negro man was stabbed in the left side of the neck, with laceration of the left jugular vein. He returned to his home and collapsed suddenly. At autopsy, thirteen hours after death, air bubbles were demonstrated in the bloody fluid of the right ventricle and the pulmonary arteries.

Case 2.—Sudden death occurred in a 26 year old white woman after fenestration of the right ear with skin grafting. The new procedure of temporary withdrawal of blood to lower the blood pressure and subsequent retransfusion was

used.[2] A sudden complete respiratory and cardiac arrest developed. An autopsy was made two hours after death. The carotid arteries were ligated. Air bubbles were seen in the cerebral arteries and in the arteries of the retina when an ophthalmoscope was used. Air bubbles were demonstrated in the left ventricle, in the aortic arch and in the carotid arteries. There was almost no air in the right ventricle.

CASE 3.—In the course of a surgical operation for torticollis of the right side of the neck of a 2 year old girl, the dome of the right pleura was slightly lacerated in a slitlike manner. The slit was closed by surgical procedure. Sudden collapse and death occurred. At autopsy, three hours after death, a diagnosis of acute emphysema of the anterior mediastinum and bilateral pneumothorax (more marked on the right side) was made. There was acute dilatation of the right cardiac ventricle. Air was demonstrated in both pleural sacs and in the mediastinum.

CASE 4.—A 27 year old white woman was found dead on the floor of the rest room of a hotel. There were no visible signs of violence. At autopsy, about eleven hours after death, the diagnosis was air embolism following attempted abortion in a case of pregnancy of two and one-half months' gestation. A lesion of the mucosa in the left lateral corner of the uterus was discovered. The embryonic sac was intact. Air was demonstrated in the right ventricle and in the pulmonary arteries. Air bubbles were also demonstrated in the ovarian veins and in the venous spaces of the uterine wall.

CASE 5.—A 47 year old white man had an infected right eyeball, which was severely damaged further during a fight. He was taken to a hospital, and there the eye was removed after anesthesia had been induced with thiopental sodium (pentothal sodium®) injected intravenously into the left arm. The widened ophthalmic veins were not ligated. The patient died suddenly after the completion of the operation. At the autopsy, nineteen hours later, air was demonstrated in the right jugular vein, the descending vena cava and the right cardiac ventricle. The resulting diagnosis of the cause of death was air embolism following enucleation of the right eye.

SUMMARY

A new device is presented to demonstrate the presence of air embolism, pneumothorax and like conditions. This device may also be used to trap gases for identification and analysis in cases of poisoning by volatile solvents and gases and might materially aid in the diagnosis of caisson disease. Five cases investigated recently are cited to illustrate the value of the device.

2. Harris, H. E., and Hale, D.: Tr. Am. Acad. Ophth. **52**:90, 1947.

Books Received

DIAGNOSTIC PROCEDURES FOR VIRUS AND RICKETTSIAL DISEASES. First edition. Price, $4. Pp. 347, with illustrations. New York: American Public Health Association, 1948.

This volume is a response to the increasing demand for a collection of laboratory methods of diagnosis of virus and rickettsial diseases in man. The authors are a committee, with Francis Thomas Jr., as chairman, of the American Public Health Association. Diagnostic procedures for the following diseases are described by investigators in virus and rickettsial infections: psittacosis, K. F. Meyer and B. Eddie; lymphogranuloma venereum, G. Rake; trachoma and inclusion blenorrhea, P. Thygesen; variola and vaccinia, R. F. Parker; influenza, G. K. Hirst; primary atypical pneumonia, A. E. Feller; mumps, J. F. Enders and J. H. Levens; poliomyelitis, J. R. Paul; encephalitis, W. M. Hammon; rabies, H. N. Johnson and T. F. Sellers; herpes simplex, T. F. McNair Scott; yellow fever, J. C. Bugher; dengue, A. B. Sabin; rickettsial diseases, N. H. Topping and J. E. Smadel. "Conceived as a manual for the laboratory worker and student the book is not intended to be a handbook of clinical diagnosis or of theoretic virology. It is a trial flight of a limited nature which the committee in future revisions will undoubtedly expand and improve as experience indicates."

BONE MARROW BIOPSY. Haematology in the Light of Sternal Puncture. By S. J. Leitner, M.D., reader in internal medicine, University of Berne (Switzerland), and deputy medical superintendent, Sanatorium for Tuberculosis, Heiligenschweni, Berne. English translation, revised and edited by C. J. C. Britton, M.D., Ch.B., D.P.H., consulting haematologist to the Prince of Wales's General Hospital, Tottenham, London, and Queen Mary's Hospital, Roehampton, and E. Neumark, M.B., B.S. (London), M.R.C.S., L.R.C.P., lecturer in pathology, St. Mary's Hospital Medical School, London. Price, $8.50. Pp. 433, with 7 plates (6 in color) and 194 text figures. New York: Grune & Stratton, 1949.

Since biopsy of marrow has become a routine procedure in many hospitals, an authoritative book on this subject should be welcome. Such a text should help the novice to gain the necessary experience and at the same time provide the expert with a reliable and comprehensive review of the many problems related to the interpretation of the findings in the disorders affecting the hemopoietic system. In general, Leitner's book covers the ground well. The technic of sternal puncture is adequately discussed, but the modern methods of hip and spinous process punctures are not mentioned. The characteristic marrow patterns are well described, and numerous case histories illustrate the diagnostic significance of biopsy of the marrow. However, the novice will regret the scarcity of colored pictures and will find it difficult to visualize important details from the black and white photomicrographs. The expert in this country will be delighted with the many aspects and references pertaining to the continental literature. He will regret that the covering of the American contributions is incomplete and thus renders this work less valuable to him than it could have been. Although this book can be recommended for the hematologic library, there is still a need for a better international text on this subject.

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